FY 1999 ANNUAL REPORT

OF

INTRAMURAL RESEARCH PROGRAM ACTIVITIES

OF THE

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

US DEPARTMENT OF HEALTH AND HUMAN SERVICES

NATIONAL INSTITUTES OF HEALTH

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REPORTING COMPONENTS

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Unit of Pharmacokinetic Studies - Susan E. Shoaf, Ph.D., Chief
Section of Brain Electrophysiology & Imaging - Daniel W. Hommer, M.D., Chief
Section of Clinical Science - David T. George, M.D., Acting Chief
Section of Cognitive Neurosciences - Herbert J. Weingartner, Ph.D., Chief
Section of Neurochemistry & Neuroendocrinology - Norman Salem Jr., Ph.D., Acting Chief

LABORATORY OF MEMBRANE BIOCHEMISTRY & BIOPHYSICS

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Unit of Metabolic Control - Richard Veech, M.D., Ph.D., Chief
Unit of Molecular Biology - Byoung-Joon Song, Ph.D., Chief
Section of Fluorescence Studies - Burton J. Litman, Ph.D., Chief
Section of Mass Spectrometry - Hee-Yong Kim, Ph.D., Chief
Section of Nuclear Magnetic Resonance - Klaus Gawrisch, Ph.D., Acting Chief

LABORATORY OF MOLECULAR & CELLULAR NEUROBIOLOGY

Section on Molecular Neuroscience - Forrest F. Weight, M.D., Acting Chief
Section on Pharmacology - Forrest F. Weight, M.D., Acting Chief
Unit on Cellular Neuropharmacology - Robert Peoples, Ph.D., Chief
Section on Physiology - Forrest F. Weight, M.D., Chief

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OFFICE OF THE CHIEF

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SECTION OF NUCLEAR MAGNETIC RESONANCE

LABORATORY OF MOLECULAR & CELLULAR NEUROBIOLOGY

SECTION ON MOLECULAR NEUROSCIENCE

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ACRONYMS

2-DG	2-deoxyglucose
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine or serotonin
AA	arachidonic acid
AAP	acetaminophen
ADHD	Attention Deficit Hyperactivity Disorder
ALDH2	aldehyde dehydrogenase
ASPD	antisocial personality disorder
ATP	adenosine 5'-triphosphate
AWS	alcohol withdrawal syndrome
CNS	central nervous system
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DHA	docosahexaenoic acid
DPH	diphenylhexatriene
DRG	dorsal root ganglion
EEG	electroencephalogram
EFA	essential fatty acids
Emax	maximal response
ENT	entorhinal cortex
ERP	event-related brain electrical potentials
Et	ethanol
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GABAA	gamma-aminobutyric acid type A
GC/MS	gas chromatography/mass spectrometry
HIPP	hippocampal
HNE	4-hydroxynonenal
HPA	hypothalamic-pituitary-adrenal
HRV	heart rate variability
HVA	homovanillic acid
IBI	inter-beat interval
IV	intravenous
LC	liquid crystalline
LDH	lactate dehydrogenase
LS	long-sleep
LVA	low voltage alpha
MDA	malondialdehyde
MHPG	3-methoxy-4-hydroxyphenyl glycol
MII	meta-rhodopsin II
MRI	magnetic resonance imaging
MTP	methyltryptophan
n-3	omega-3
nACh	nicotinic acetylcholine
NER	nucleotide excision repair
NIDDM	noninsulin-dependent diabetes mellitus
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
OCD	obsessive-compulsive disorder
PC	phosphatidylcholines
PDE	phosphodiesterase
PET	positron emission tomography
PKA	protein kinase A
PKC	protein kinase C
PNS	parasympathetic nervous system
PS	phosphatidylserine
PTSD	post traumatic stress disorder
SAD	seasonal affective disorder
sMRI	structural magnetic resonance imaging
SNPs	single nucleotide polymorphisms
SS	short-sleep
SSCP	single-strand conformational polymorphism
SVZa	anterior subventricular zone
TCA	tricarboxcyclic acid
TDO2	tryptophan 2,3-dioxygenase
TDT	transmission/disequilibrium test
TEA	tetraethylammonium
TPH	tryptophan hydroxylase
TPQ	Tridimensional Personality Questionnaire

FY 1999 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1998 - 30 SEPTEMBER 1999)

LABORATORY OF CLINICAL STUDIES

DANIEL W. HOMMER, M.D., ACTING CHIEF

DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE & ALCOHOLISM
NATIONAL INSTITUTES OF HEALTH

SYNOPSIS
LABORATORY OF CLINICAL STUDIES
1 OCTOBER 1998 - 30 SEPTEMBER 1999

INTRODUCTION

During FY 1999, the 11-bed ward of the laboratory continued to be one of the busiest inpatient services in the Clinical Center. Long-term, projects were expanded in the area of brain imaging, to develop new serotonin receptor ligands, to use functional MRI to explore the functional neuroanatomy of reward and punishment and to develop techniques for automated measurement of neuroanatomically defined brain structures. The unique brain imaging resources and expertise at the NIH Clinical Center make these efforts particularly important and rewarding.

UNIT OF CLINICAL AND BIOCHEMICAL PHARMACOLOGY

Our laboratory is investigating the mechanisms underlying alcohol-related neurotoxicity, as well as the effects of this toxicity on the autonomic nervous system. We have shown that the activity of a class of calcium-activated proteases called calpains, which regulate neuronal cellular signaling by degrading proteins, are strongly affected by alcohol exposure at concentrations that are clinically relevant. The activity of these proteases has been implicated in mediating the toxic effects of agents that activate NMDA receptors, which are thought to be overactive during the alcohol withdrawal state in mammals. We have found that alcohol exposure strongly increases the cytoplasmic immunofluorescent signal associated with one of these proteins, m-calpain, while not altering total cellular protein levels. We hypothesize that this alteration in cellular location may underlie the decrease in protease activity that we found in previous work. We are also continuing our work to identify what proteins interact with these proteases, and how their interaction modulates protease function in the presence and absence of alcohol. As part of this work, we have also developed and refined cytotoxicity assays, which are being used to quantify the toxic effects of alcohol and to screen compounds which may have neuroprotective effects.

In higher mammals, it appears that the activity of the parasympathetic nervous system (PNS) depends on the feedback loop operating between peripheral and central 5HT3 receptor-mediated control of acetylcholine release. Changes in PNS activity are reflected in alterations in the dynamics of heart rate variability. Working with primates, we have shown that ketamine greatly increases cardiac signal complexity, and that this effect is attentuated by ondansetron, a 5HT3 antagonist, suggesting that heart rate variability may partially depend on intact serotonergic functioning. We are now investigating, in a primate model, whether one exposure to high intoxicating levels of alcohol in alcohol-naive subjects may permanently alter cardiac signal complexity. We are also interested to learn if exposure to ketamine may result in permanent changes in heart rate regulation. These two agents share the same NMDA-receptor antagonist properties. It is hypothesized that both agents may exhibit a dose-related toxicity to brain stem neurons involved in heart rate regulation.

UNIT OF PHARMACOKINETIC STUDIES

Research efforts of the Unit of Pharmacokinetic Studies involve the use of various specific serotonin receptor antagonists, labeled with positron-emitting radioisotopes, to compare the differences in serotonin receptor concentration between alcoholic and normal individuals in both non-human primate and human populations. Research concerning the determination of the kinetics of new therapeutic agents, that are being evaluated as treatments in non-human primates, for anxiety-mediated and/or stress and non-stress conditions is also being pursued.

Another area of investigation is the role of alcohol dehydrogenase genotype and glucose metabolism in ethanol elimination. Ethanol elimination rates will be compared in individuals with different ethnic backgrounds (European Caucasian, African-American, Hispanic and Asian) but similar alcohol dehydrogenase genotypes. Men and women will be given doses of ethanol, based on total body water, to account for differences in ethanol distribution between the sexes and ethnic groups. The role of a

second enzyme pathway, in the elimination of ethanol, is being investigated by developing a two-compartment open pharmacokinetic model that incorporates dual Michaelis Menten based elimination pathways. Ethanol will be given to both men and women intravenously, to avoid absorption differences, and doses will be given as g/l total body water to account for differences in ethanol distribution.

SECTION OF BRAIN ELECTROPHYSIOLOGY AND IMAGING

Investigators in the Section of Brain Electrophysiology and Imaging conduct sophisticated electrophysiological, neuropsychological and brain imaging studies on alcoholics, individuals at risk and carefully matched controls. The lack of an acceptable method for determining statistical significance of differences in brain images derived from functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) studies has been a major problem for researchers in these areas. Over the past several years, we have made significant progress in applying rigorous statistical methods, based on a Gaussian random field model, to the analysis of image data. In addition, work on other methods have been developed, for determining the statistical significance of differences observed in arbitrary regions of group average images. These include methods of spatial frequency decomposition as well as wavelet analysis. We have compared the relative merits and shortcomings of these methods with Gaussian random field based techniques. Gaussian random field based techniques are the most conservative statistically and give the most precise spatial localization; they are particularly suited to analysis of FDG PET data. We have developed an approach to extend Gaussian random field based techniques to the analysis of fMRI time series data. This technique is particularly useful in fMRI studies of drug effects or emotions where it is difficult to collect temporally independent scans of functional activity.

Work on the development of advanced image analysis and coregistration for PET, CT, structural and fMRI has continued. Methods to achieve the 3-D registration of PET images with structural MRI have been developed as have techniques for the automated detection of midsagittal lines or planes. Using these techniques, we can now identify structures as small as the head of the caudate nucleus or nucleus accumbens on coregistered PET and MRI scans.

Using these analysis techniques, we have been able to demonstrate significant differences in glucose metabolism in the brains of normal controls and individuals that have developed anti-social behavior following a serious closed head injury. Analysis was performed using both absolute-pixel glucose uptake values and means-adjusted pixel values obtained by subtracting the overall mean brain glucose uptake from each pixel glucose uptake. Both mean-adjusted and absolute glucose uptake produced similar results. Head injured subjects had significantly lower glucose uptake in the posterior orbital cortex bilaterally, in the right caudate, in the right dorsal thalamus and in mesial superior frontal cortex. Glucose uptake in the caudate, thalamus and superior frontal cortex were significantly correlated with ratings of aggressive behavior developed after the injury. In addition, we have coregistered these PET images with subjects' structural MRI (sMRI) to show that the regional reduction in glucose uptake corresponds nearly perfectly to the regions of tissue loss in the caudate and thalamus.

Fully automated segmentation techniques have been developed for use on T-1 weighted MRI. These techniques allow for the automated labeling of cerebrospinal fluid (CSF) and white and gray matter regions in sMRI data. We have applied these techniques and found evidence for selective reduction in the volume of the hippocampus among alcoholics. In addition, we have found both gender and laterality differences in this structure, among both controls and alcoholics. We have also demonstrated significantly greater reductions in brain gray matter volume among alcoholic women compared to alcoholic men. Techniques to expand this type of analysis to other brain regions are now being developed. An automated method of measuring the volume of the medial prefrontal cortex is now being tested for reliability and validity.

Over the past year we have developed fMRI techniques to measure changes in cerebral blood flow/blood volume that occur during operant tasks which are under either appetive or aversive control. In healthy normal subjects, we have found that both reward and punishment increases activity in the mesial frontal cortex (BA 24 and 32), the dorsal thalamus and caudate nucleus. The magnitude of activation appears to be greater during expectation of punishment than during expectation of reward. Expectation of reward activates the nucleus accumbens while expectation of punishment does not. These studies are being extended to recovered alcoholics in order to determine if alcoholics show a differential sensitivity to reward and punishment compared to non-alcoholic controls.

SECTION OF CLINICAL SCIENCE

The major research objectives in the Section of Clinical Science are to utilize pharmacological challenge paradigms, PET studies, and CSF metabolite determinations to understand the etiology of alcoholism and violent behavior; to describe and understand the interaction between alcohol and acts of domestic violence; to characterize the concept of "losing control" as it relates to acts of domestic violence and alcoholic drinking; to introduce new pharmacological interventions to treat perpetrators of domestic violence; to investigate the effect of glucoprivic stress on hypothalamic-pituitary-adrenal (HPA) axis in patients with alcoholism; and, to explore the effect of protracted alcohol withdrawal on the HPA axis function.

The majority of our research this year has been directed toward understanding the psychological and biological features of individuals who initiate acts of domestic violence. Domestic violence is a major problem in the United States; it is estimated that approximately 30% of all women will be assaulted by their spouse/significant other at some time in their lives. Of special interest, 70% of the perpetrators have an alcohol problem. To date, the majority of studies have focused on psychosocial issues; minimal emphasis has been given to biological factors that could contribute to the violence.

We have studied approximately 50 perpetrators of domestic violence and 100 non-violent controls, both with and without alcoholism. Based on our clinical evaluations of perpetrators and the results from our lactate infusions, we formulated the hypothesis that some perpetrators have an abnormality in their ability to inhibit fear-induced aggression. Using the animal literature as well as the results from our PET study, comparing central glucose utilization in perpetrators to non-violent controls, we have developed a model outlining the neuropathways that potentially could be involved in the mediation of fear-induced aggression.

Approximately 50% of our perpetrators were exposed to violence growing-up. This led to the hypothesis that exposure to violence could alter the central noradrenergic function of some perpetrators. Previous studies have shown that subjects with post traumatic stress disorder (PTSD) have disturbances in norepinephrine metabolism that predispose them to over-react to stimuli which they perceived as threatening. To explore this possibility, we employed the alpha-2 adrenergic antagonist, yohimbine, to study adrenergic receptor function in perpetrators and non-violent controls. The study has been completed and the results are being analyzed.

Previous studies on aggression have shown a negative correlation between impulsive acts of aggression and the CSF metabolite of serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA). To test the hypothesis, i.e., 5HT has a role in the modulation of fear-induced aggression, we measured CSF 5HIAA in perpetrators and non-violent controls. Pair-wise comparisons revealed perpetrators without alcoholism had lower concentrations of CSF 5HIAA than perpetrators with alcoholism, non-violent alcoholics and healthy comparison subjects. These results suggest that 5HT may have a role in modulating pathways that mediate fear-induced aggression. The fact that perpetrators with alcoholism had higher 5HIAA concentrations than perpetrators without alcoholism suggests that there may be a prolonged effect of alcohol withdrawal. This possibility is supported by studies showing that CSF 5HIAA can remain elevated for weeks following alcohol cessation.

Long-term effects of alcohol withdrawal on hypothalamic function were studied by administering the glucoprivic agent, 2-deoxyglucose (2-DG), to alcoholics who had been alcohol-free for at least 6 months and healthy comparison subjects. This research was initiated in response to our previous study showing that alcoholics, who had been abstinent for 3 weeks, had an exaggerated ACTH response to 2-DG when compared to controls. The fact that we found no statistical difference in 2-DG-induced ACTH response between long-term abstinent alcoholics and controls suggests that our earlier findings were the result of acute withdrawal and did not represent trait phenomena.

SECTION OF COGNITIVE NEUROSCIENCES

Detoxified alcoholics demonstrate a specific type of cognitive impairment which is expressed as a failure to inhibit errors in learning, remembering and attention and an associated impairment in ability to track the

source of remembered knowledge. This is evident under unstructured information processing conditions but not when they are directly asked to monitor and evaluate their own cognitive performance. Polydrug abusers demonstrate a similar type of cognitive deficit. The cognitive style of alcoholics and polydrug abusers are more likely to be defined by external stimuli rather than conceptually (internally) driven cognitive processing that characterizes the information processing style of normal volunteers. Performance of alcoholics on tasks that require reflective functions, such as explicit remembering and inhibition of intrusions, is not correlated with CSF measures of the neurotransmitter metabolites, 5HIAA and homovanillic acid (HVA), nor neuroanatomical variables as assessed by brain imaging methods. We are currently exploring whether the "cognitive style" used by alcoholics is a risk factor for developing alcoholism. Ongoing studies of children at risk for alcoholism and genetic-twin studies of alcoholics will allow us to test this hypothesis.

The cognitive changes associated with normal aging (and depression) are qualitatively different from the cognitive impairments apparent in detoxified alcoholics. Large, highly reliable and generally linear, aging-related declines in cognitive function are apparent for all of the cognitive domains cited above, and, no single proposed model of cognitive aging accounts for the broad and robust changes in cognitive function associated with aging. The pattern of aging-related changes in cognition is discriminable from those expressed in detoxified alcoholics as well as those in early Alzheimer's disease.

Benzodiazepines potentate preexisting impairments in reflective (control) cognitive operations in detoxified alcoholics and polydrug abusing patients. This is expressed as a selective impairment in ability to suppress intrusions, monitor the source of remembered knowledge, attend to information without the benefit of orienting cues, encoding functions under unstructured processing conditions and the perception of ambiguous information. The effects of benzodiazepines on attention are similar to those produced by alcohol and are the opposite of attentional effects associated with aging. Furthermore, unlike normal volunteers, detoxified alcoholics demonstrate a robust qualitative shift in how they think about 'standard' stimuli.

The effect of alcohol on cognitive functions is similar to that observed following the administration of a benzodiazepine and different from the response to other classes of drugs, i.e., cholinergic antagonists. Effects of other benzodiazepines, such as alprazolam and adinazolam, produce similar dose-dependent sedative and cognitive effects that simulate the cognitive deficits expressed in untreated amnestic patients. The selective cognitive impairing effects of benzodiazepines are, at least, partially independent of their sedative effects. The effect of the anesthetic, ketamine, an antagonist of the N-methyl-D-aspartate (NMDA)-type glutamate receptor (thought to be involved in memory consolidation) mimics cognitive changes expressed in normal aging. It has been shown that this receptor can be altered by alcohol. Paradoxically, subjects that acquire knowledge prior to the administration of a benzodiazepine, such as triazolam, demonstrate facilitation in recall of that information (compared to recall tested under placebo conditions).

The profile of highly specific effects of benzodiazepine (and alcohol) on reflective functions is thought to be important in understanding patterns of uncontrolled drinking and may provide information on the stimulus discriminative (and reinforcing) properties of these drugs in terms of the selective aspects of autobiographical memory that are elicited under drug in contrast to undrugged conditions. The specific types of cognitive changes that are induced by benzodiazepines (and alcohol) are currently being developed to provide models of the cognitive changes apparent not only in the amnestic alcoholic, but also in nominally unimpaired, detoxified alcoholics; knowledge that can be used to more effectively treat patients with alcoholism.

SECTION ON NEUROCHEMISTRY AND NEUROENDOCRINOLOGY

The Section on Neurochemistry and Neuroendocrinology has continued research on biochemical concomitants of violent behavior in alcoholics and on variables associated with increased vulnerability of developing alcoholism-related behavior. The major focus of this research has remained the serotonergic system. Interesting insights have been gained into regulation of serotonergic neuronal networks, developmental and genetic influences on serotonin functions and serotonergic regulation of metabolism and excessive alcohol consumption.

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Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00002-07 LCS

Title: EYE MOVEMENTS IN ALCOHOLISM AND INDIVIDUALS AT RISK FOR ALCOHOLISM

Staff Years: 2.25

Principal Investigator: Hommer DW, MD (BEI, LCS, NIAAA)

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Other Collaborators: None

Sample Type: Human subjects

Keywords: behavioral research, neurosciences, electrophysiology/EEG

Summary: The study of human eye movements provides an extremely useful approach to the examination of a variety of cognitive functions. It is obvious that the latency and goal of saccadic eye movements are related to attention. What is not so obvious is that other aspects of cognition such as short-term memory, preparatory set and inhibition of context inappropriate responses can also be assessed using eye movement techniques. Short-term memory, preparatory set and inhibition of context inappropriate responses constitute core functions of the prefrontal cortex, the brain region most involved in the control of higher order cognitive processes. We have used a number of different tasks to elicit saccades, including Go/No Go and delayed response tasks. These tasks allow us to independently assess core functions of the prefrontal cortex by measuring the accuracy and latency of memory guided saccades, as well as the frequency of context inappropriate saccades that should be inhibited. Using these tasks we have demonstrated that schizophrenics are impaired in all core aspects of prefrontal cortex function while children with Attention Deficit Hyperactivity Disorder (ADHD) are impaired in only their ability to inhibit context inappropriate saccades. Similarly, adult alcoholics have difficulty inhibiting context inappropriate saccades. The smooth pursuit eye movements of alcoholics are completely normal; however, preliminary evidence suggests that cerebellar volume, measured by MRI, correlates with smooth pursuit performance.

RESEARCH HIGHLIGHTS: This research allow us to independently assess core functions of the prefrontal cortex by measuring the accuracy and latency of memory guided saccades, as well as the frequency of context inappropriate saccades that should be inhibited. Using these tasks, we have demonstrated that schizophrenics are impaired in three core aspects of prefrontal cortex function while children with Attention Deficit Hyperactivity Disorder (ADHD) are impaired in only their ability to inhibit context inappropriate saccades. Similarly, adult alcoholics have difficulty inhibiting context inappropriate saccades.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The study of human eye movements provides an extremely useful approach to the examination of a variety of cognitive functions. It has an advantage over other methods of measuring cognitive function since it is completely non-verbal and thus free of any cultural bias.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00059-08 LCS

Title: DETERMINANTS OF COGNITIVE DYSFUNCTIONS IN NEUROPSYCHIATRIC DISORDERS

Staff Years: 3.75

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Sample Type: Human subjects

Keywords: cognitive deficits, aging, reflective functions, attention, learning and memory, dementia, amnesia, alcohol, benzodiazepines, PET/MRI

Summary: The aim of this project is to uncover, assess and contrast mechanisms that are responsible for impairments in cognitive functioning in different forms of neuropsychiatric disorders, emphasizing syndromes that are associated with alcohol abuse. A cognitive neuroscience prospective along with brain imaging and neuropharmacological methods are used to consider how disordered cognitive function in alcohol and drug-abusing subjects is involved in the development and maintenance of alcohol addiction and abuse. In substance abusing subjects, findings suggest that there are selective impairments in strategic use of cognitive planning and evaluative functions. These may be expressed in the ability to appropriately: inhibit behaviors, use conceptually-driven plans in contrast to data or stimulus-driven cognitive operations and/or use meta-cognitive processes such as monitoring the source of what is remembered. A developmental perspective has also been used to consider how reflective functions are acquired (nature vs nurture) and whether interventions can be helpful in teaching reflective functions (i.e., as part of an educational process or in targeted behavioral therapies). Our goals have been to assess and evaluate: whether these deficits are independent of other aspects of impaired cognitive function in these subjects; the neurobiological and behavioral mechanisms of impairments in reflective cognitive operations; whether these deficits are relatively unique to alcoholics (i.e., are not observed in patients with other forms of neuropsychiatric disorders); conditions that potentate and attenuate underlying impairments in reflective-inhibitory cognitive function; clinical and therapeutic implications of this type of cognitive impairment; and, impairments in cognition that may be antecedents to the development of alcoholism. New initiatives are planned to consider alternative types of training that could be used to cultivate reflective function.

This is the terminal year for this project. New protocols will be written to continue to study normal and impaired reflective cognitive functions (executive functions).

RESEARCH HIGHLIGHTS: We have described, in a model, problems associated with substance abuse (from a cognitive perspective). Problems associated with dependence, vulnerability and relapse are interpreted in cognitive terms. Our earlier research efforts, as well as those of others, are used to support this model.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: This research is designed to provide new knowledge and approaches to further understanding of the causes, consequences and treatment of substance abuse. This research is helpful in characterizing how cognitive functions may fail in different populations of patients, particularly those that abuse alcohol. The cognitive "style" of alcoholics may be important in determining the maladaptive behaviors that maintain patterns of alcohol abuse and addiction.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00060-08 LCS

Title: DRUG EFFECTS ON MEMORY AND RELATED COGNITIVE FUNCTIONS

Principal Investigator: Weingartner HJ, PhD (CN, LCS, NIAAA)

Summary: TERMINATED

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00061-08 LCS

Title: CEREBRAL STRUCTURAL AND METABOLIC CORRELATES OF AGGRESSIVE OR ADDICTIVE BEHAVIOR

Staff Years: 5

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Other Collaborators: None

Sample Type: Human subjects

Keywords: neurosciences, imaging, PET, MRI

Summary: This research is designed to determine neuroanatomical and neurochemical correlates of addictive and aggressive/impulsive behavior in human subjects. The principal focus of these studies is the measurement and correlation of regional cerebral glucose metabolic activity using positron emission tomography (PET), brain volumes using magnetic resonance imaging (MRI) and cerebrospinal fluid metabolites, and measures of impulsive/aggressive behavior and excessive alcohol consumption. We collected full volumetric T-1 weighted MR images using a 1.5 T scanner to measure intra-cranial volumes in 68 alcoholics (38 males and 30 females) and 38 healthy, non-alcoholic comparison subjects (19 males and 19 females). An automated segmentation program was used to divide the intra-cranial contents into CSF, gray and white matter (Human Brain Mapping, 5:194-205, 1997). We also measured the cross-sectional area of the corpus callosum. Since women have significantly smaller intra-cranial volumes than men do, the genders were analyzed separately. Women alcoholics showed significant reductions in cerebral gray and white matter volumes compared to healthy nonalcoholic women, as well as significant increases in ventricular and sulcal CSF. Neither gray matter volume nor corpus callosum area differed significantly between alcoholic and non-alcoholic men but alcoholic men did have significantly more sulcal CSF than non-alcoholic men. Women appear to be more susceptible to alcohol-associated brain damage than men. In contrast to the significant reductions in cerebral volume of alcoholics compared to controls, there was no significant difference in the cerebellar volume of the two groups.

RESEARCH HIGHLIGHTS: Women alcoholics showed significant reductions in cerebral gray and white matter volumes compared to healthy non-alcoholic women, as well as significant increases in ventricular and sulcal CSF. Neither gray matter volume nor corpus callosum area differed significantly between alcoholic and non-alcoholic men but alcoholic men did have significantly more sulcal CSF than non-alcoholic men. Women appear to be more susceptible to alcoholism associated brain damage than men. In contrast to the significant reductions in cerebral volume of alcoholics compared to controls, we found no significant difference in the cerebellar volume between alcoholics and controls.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: This work provides the first unequivocal demonstration of greater alcohol-induced brain damage among women as compared to men. It also shows that brain volume reduction among alcoholics occurs early in the course of the disease, i.e., before 30 years of age, suggesting that early intervention is critical.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00063-08 LCS

Title: EEG STUDIES OF ELECTROMOTIVE GENERATORS AFFECTED BY ALCOHOL

Staff Years: 2

Principal Investigator: Hommer DW, MD (BEI, LCS, NIAAA)

Other Personnel: Adams CW, PhD (BEI, LCS, NIAAA)
Davis EZ (BEI, LCS, NIAAA)
Rio DE, PhD (BEI, LCS, NIAAA)

NIH Collaborators: Parma-Dotson D (CN, LCS, NIAAA)

Other Collaborators: Rohrbaugh J, PhD (Psychiatry, Washington University)

Sample Type: Human subjects

Keywords: behavioral research, neurosciences, electrophysiology/EEG

Summary: The electrophysiological arm of the Section of Brain Imaging and Electrophysiology continues to make significant methodological advances in stimulus generation, control and presentation within multiple sense modalities. We can now present any sensory event that can be digitally stored (including sounds, visual images, tactile patterns, etc.) in complete synchrony with the continuous EEG acquisition. We have also written C code to dynamically update the identity of a specified stimulus in a predefined sequence. The update allows each pre-specified stimulus sequence to be uniquely altered based on the response history of each individual. All subject responses can be logged at any point within the sequence of stimulus presentation; not just at predefined "response intervals." This enables analysis of complex patterns of responding that may be useful in characterizing groups of individuals with different response profiles in tasks of varying cognitive demand. Additionally, this code has been modified for use in fMRI so identical versions of tasks in the electrophysiology and fMRI/PET settings may be used. We are actively pursuing analytic strategies to co-register the electrode array data with that of structural MRI and functional blood flow data within a common coordinated system. Using recordings obtained from spatially dense arrays of electrodes placed on the head, the fine temporal information obtained from EEG/ERP techniques can be combined with the fine spatial information of imaging modalities such as PET and fMRI to construct true spatio-temporal models of neural networks underlying cognitive phenomena. It is necessary to augment the electrophysiological data collected using multisensory selective attention tasks with imaging data in order to more precisely visualize and characterize the multisensory processing areas mentioned above. The use of imaging modalities will allow verification of activation in these multisensory areas of the brain and the use of EEG/ERP techniques enable us to determine when these areas become activated. Thus a model of the spatio-temporal dynamics of the neural network that underlies selective processing of stimuli across sensory modalities can be produced. Once a model of normal multisensory selective processing has been validated, we can look at different patterns of disruption in individuals exhibiting impulse control problems to aid in identifying the source of these disruptions.

RESEARCH HIGHLIGHTS: The electrophysiological arm of the Section of Brain Imaging and Electrophysiology continues to make significant methodological advances in stimulus generation, control and presentation within multiple sense modalities. Any sensory event that can be digitally stored (including sounds, visual images, tactile patterns, etc.) can now be presented in complete synchrony with the continuous EEG acquisition. We have also written C code to dynamically update the identity of a specified stimulus in a predefined sequence. This updating allows each pre-specified stimulus sequence to be uniquely altered based on the response history of each subject. All subject responses can be logged at any point within the sequence of stimulus presentation; not just at predefined "response intervals." This enables examination of complex patterns of responding that may be useful in characterizing groups of individuals with different response profiles in tasks of varying cognitive demand.

This code has also been modified for use in fMRI so identical versions of tasks can be run in both the electrophysiology and fMRI/PET settings.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: This allows us to test experimental designs in the electrophysiology lab before moving them into an imaging setting which saves considerable time and expense.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00064-08 LCS

Title: ANALYSIS OF BRAIN IMAGES

Staff Years: 1

Principal Investigator: Rio DE, PhD (BEI, LCS, NIAAA)

Other Personnel: Kerich M (BEI, LCS, NIAAA)
Hommer DW, MD (BEI, LCS, NIAAA)
Williams WA, MD (BEI, LCS, NIAAA)

NIH Collaborators: Rawlings RR, MS (CN, LCS, NIAAA)

Other Collaborators: Momenan R, PhD (MedData)
Woltz LA, PhD (Synergy Research)

Sample Type: Human subjects

Keywords: neurosciences, PET/MRI

Summary: Traditional methods of analyzing image data from PET, MRI and fMRI have proven only partially successful. This is due, in part, to the inherent biological variability, physical limitation of the acquisition instrumentation and mathematical algorithms applied to reconstruct the image data, but it also reflects the inadequacy of the computational, mathematical and statistical methods employed in analyzing these data. Image data acquired by PET and MRI have numerous sources of distortion. Depending on the imaging modality, these appear as spatial distortions, decreases in signal-to-noise ratio, modification of image values and increased spatial correlation. PET and MRI data can be "improved" by using appropriate models to restore and analyze the reconstructed image. Methods in the spatial domain, using the theory of Gaussian random fields, and Fourier or frequency domain have been developed for analysis of PET and fMRI. In order to evaluate these models, simulated PET brain intensity data and PET and MRI brain shape data have been created using empirically measured image characteristics for PET and MRI. In particular, Monte Carlo techniques have been developed to create groups of PET data with known attributes and specific group differences. The control of signal and noise associated with these models allows us to evaluate the effect of geometric distortions and sensitivity of identification of localized statistically significant differences between the groups. In the case of geometric models, it is possible to create 3-D brain (or skull) shapes with known noise, to evaluate the limitations of re-scaling of PET images across subjects to a given standard and registration of MRI and PET images for the same subject. These simulations are being used to study two related areas. Statistical techniques are being researched for both geometric and gray scale values of PET, MRI and fMRI data, and the precision of multimodality 3-D superposition of functional and structural images obtained for PET and MRI data, especially when the data are sparse or have known symmetries. With respect to 3-D superposition, specific validation of these techniques has been initiated. A mechanical head-held fiducial system has been developed which allows marker-based registration of multimoldality images, test-retest images and images which could not otherwise be registered by their intrinsic information. Incorporated into existing experiments, this hardware will provide an alternative to brain landmark registration and help validate existing algorithms.

RESEARCH HIGHLIGHTS: We have continued to investigate and develop a mechanical head-held fiducial system. We are also working with Nuclear Medicine and the MRI Center to implement the system in initial test trial in studies where reliable positioning of patient is critical, but patient is not immobilized.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The fiducial system will have uses in many studies using PET and MRI imaging. Validation of our post hoc methods to orient and identify anatomical regions of the brain.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00065-08 LCS

Title: SEMIAUTOMATED METHODS OF SEGMENTATION OF BRAIN IMAGES

Staff Years: 0.58

Principal Investigator: Rio DE, PhD (BEI, LCS, NIAAA)

Other Personnel: Hommer DW, MD (BEI, LCS, NIAAA)

NIH Collaborators: None

Other Collaborators: Momenan R, PhD (MedData)
Woltz LA, PhD (Synergy Research)

Sample Type: Interview, questionnaires or surveys only

Keywords: neurosciences, imaging, PET, MRI

Summary: Establishing associations between structure and function among various areas of the brain is an important step in identifying neurological mechanisms in both normal and disease states. The pursuit of this goal requires geometrical co-registration of digital images in two types of major applications: (1) data fusion, where brain images from the same subject are acquired by different modalities and (2) data comparison, to detect significant differences between subject groups in images acquired by the same modality. The first application requires identification and delineation, i.e., segmentation, of distinct areas and landmarks in the brain. A procedure based on dynamic clustering and region growing algorithms has been developed that segments T1-weighted MR images into regions of CSF, gray and white brain matter. This technique is currently being applied to automatic/semi-automatic identification of the following specific brain structures: cerebellum, good automated detection with operator allowed editing; caudate, limited success at automated detection. This technique has been found to be of value in clinical research projects that use morphometry as an important measurement tool along with other physiological and sociological measures. Associated with the above developments, a technique based on a previously investigated algorithm has been further developed for automatic detection of axis of symmetry (intra-hemispheric fissure) in PET and MR images. This algorithm is based on the correlation of similar, but possible phased, signals in the Fourier domain between values of corresponding spatial points. Using continuity conditions, this has been extended to 3-D. This technique allows us to first identify the fissure in brain images and then, using previously developed algorithms, to orient the brain images of all subjects in the same vertical manner. This is helpful in obtaining consistent volumetric measures of brain structures that have traditionally been viewed as 2-D cross-sections. In addition, brain image visual presentation, for both clinical review and editing purposes, and landmark orientation, for multimodality registration, is also made more consistent. For the second (within-modality) application, the gray-level information itself can be employed for image registration without the need for segmentation. A multiscale registration procedure has been implemented that determines parameters of a general 3-D affine transformation (translation, rotation about an arbitrary center, anisotropic scaling and skewing) between volumes to be registered that minimizes the average squared gray-level difference between corresponding voxels. Successful registration of PET images achieving homogeneous registration variance across the entire brain section has been achieved for both within and between subject analyses. This technique is now being used routinely in many of our clinical investigation. Furthermore this method is also being used for co-registration of long time series (e.g., 199 time points) of volumes acquired in structural MRI or fMRI studies.

RESEARCH HIGHLIGHTS: Segmentation methods developed were used to analyze data reported in the publication by Agartz A, Momenan R, Rawlings RR, Kerich MJ, Hommer DW: Hippocampal volumes in patients with alcohol dependence. Archives of General Psychiatry, 56:356-363,1999.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Reliable, reproducible segmentation methods were developed and applied. May ultimately lead to better understanding of the effects of alcoholism.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00068-08 LCS

Title: CNS SEROTONIN AND THE REGULATION OF PERIPHERAL GLUCOSE METABOLISM

Staff Years: 0.1

Principal Investigator: Eskay R, PhD (NN, LCS, NIAAA)

Other Personnel: None

NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: behavioral research, neurosciences, mental health research, nutritional disorders, endocrine system, CNS, cravings/addictions, cognitive deficits

Summary: Increased alcohol preference and consumption, depressed mood and impulsive aggression are thought to be linked, in part, through decreased central serotoninergic (5HT) activity. In agreement with this postulate, certain agents which increase central serotonergic neurotransmission (5HT precursors, 5HT uptake inhibitors, 5HT receptor antagonists) attenuate ethanol intake, improve memory function in intoxicated patients and may improve memory function in patients with Korsakoff's psychosis. Recently, a possible pattern of atypical glucose metabolism has emerged in alcohol abusing, impulsive, violent offenders with apparent central serotonergic dysfunction. In a group of impulsive offenders, hypoglycemia was possibly due to increased insulin secretion. It is possible that a relative hypoglycemic state or abnormal insulin levels may contribute to violent, aggressive behavior in violent offenders with apparently reduced central 5HT activity; however, this hypothesis awaits substantially more scientific verification. Animal studies demonstrating a cause and effect relationship between altered central serotonin activity and abnormal glucose metabolism have not been performed. However, overwhelming evidence suggests that appropriate glucose levels is maintained through complex feedback involving the sympathoadrenalmedullary system through the glucose mobilizing hormone, epinephrine, and the endocrine pancreas via insulin and glucagon secretion. Finally, we have established that serotonergic neurotransmission, particularly events that are mediated via the central 5HT1A receptor subtype, is a significant mediator of normal glucose homeostasis. Activation of central 5HT1A receptors increases circulating levels of insulin, glucagon and glucose through a physiological mechanism that requires permissive levels of glucocorticoids and a hormone signal of unknown origin that is released from the anterior pituitary gland. Additional experimental research was not performed during the current fiscal year; however, data analysis and manuscript preparation was carried out.

RESEARCH HIGHLIGHTS: The results of this study indicate that the central serotonin neurotransmitter system plays a significant role in the regulation of peripheral glucose metabolism. Of the many serotonin receptor subtypes in the brain, activation of the 1A subtype is the most potent stimulator of pancreatic hormone secretion.

SIGNIFICANCE TO BIOMED RESEARCH & PROGRAM: Altered central serotonin (5HT) activity has been found to be associated with obesity, eating disorders and craving. Since all of these conditions can be considered dysfunctional nutrient intake or disposition states, our finding suggest that the central 5HT1A receptor subtype may play an important role in the onset or maintenance of the aforementioned disorders.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00077-06 LCS

Title: IMPAIRED CNS SEROTONIN ACTIVITY: TOLERANCE, NEUROIMAGING, AND NEUROANATOMY

Staff Years: 1.5

Principal Investigator: Higley JD, PhD (NN, LCS, NIAAA)

Other Personnel: Hurley AC, BS (NN, LCS, NIAAA)
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Bennett AJ, PhD (NN, LCS, NIAAA)
Flory GS, BS (NN, LCS, NIAAA)
Goldstein MA, BS (NN, LCS, NIAAA)
Graham ST (NN, LCS, NIAAA)
Lindell S, BS (NN, LCS, NIAAA)
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Tsai T, BS (LCE, NICHD)

Other Collaborators: Lopez J, MD (Mental Health Research Institute, University of Michigan)
Watson S, MD (Mental Health Research Institute, University of Michigan)

Sample Type: Neither human subjects nor human tissues

Keywords: social behavior, violence, tolerance, neurosciences, neuroanatomy, genetics (nonhuman), alcohol and alcoholism, neuroimaging, serotonin

Summary: During the past year, our investigations continued on central nervous system (CNS) serotonin correlates of low cerebrospinal fluid (CSF) 5HIAA and tolerance to the intoxicating effects of alcohol. This research included the following:

To evaluate a new measure of intoxication and to further investigate the relationship between CNS serotonin activity and behavioral tolerance to ethanol, CSF concentrations of 5HIAA and behavior ratings were obtained from three cohorts of alcohol-naive adolescent rhesus macaque monkeys (N=60; 23 males, 37 females). Subjects were reared in one of three conditions: peer only-, surrogate peer-, or mother-reared. One month after baseline CSF samples were obtained, subjects were given an IV bolus of 2.1 grams/kg (males) or 2.0 grams/kg (females) ethanol, placed into a clear Plexiglas box suspended from the ceiling in an enclosure, and the latency to escape was recorded. Animals were also rated for degree of intoxication and frequency of aggressive behaviors during a 35-minute observation period. Results showed that following the administration of ethanol, the latency to escape from the box was positively correlated with subjective ratings for intoxication. Baseline CSF 5HIAA concentrations were positively correlated with escape latency and, another measure of intoxication, the number of times subjects fell. CSF 5HIAA was positively correlated with blood alcohol concentration 5 and10 minutes after the beginning of the infusion. This study shows that in alcohol naive monkeys, objective measures of intoxication are positively correlated with a subjective rating for intoxication, and the degree of

intoxication following a standardized alcohol dosage is correlated with CNS serotonin functioning. Our data confirm and extend the finding that low CSF 5HIAA is predictive of increased intrinsic tolerance to the intoxicating effects of ethanol. These data also suggest that low central serotonin turnover may expedite the metabolism of ethanol in non-human primates.

In a second series of studies, we analyzed the effect of alcohol on CNS functioning and the relationship between this effect and future alcohol consumption. Results showed that relative to baseline, concentrations of plasma cortisol and ACTH and CSF MHPG, HVA and 5HIAA (the major metabolites of norepinephrine, dopamine and serotonin, respectively) were increased by the IV alcohol infusion. Baseline 5HIAA, MHPG, HVA, ACTH and cortisol, as well as IV alcohol-induced MHPG, HVA and ACTH all predicted future voluntary alcohol consumption. MHPG, HVA, 5HIAA, ACTH and cortisol concentrations were highly correlated across baseline and IV alcohol conditions, demonstrating that individual differences in neurotransmitter levels were stable and trait-like and that baseline CSF 5HIAA concentrations are predictive of monoamine and HPA functioning following alcohol consumption. These findings suggest that alcohol has strong effects on CNS neurotransmission and hormonal functioning and that this response may be important in predicting, and possibly mediating, alcohol consumption.

CSF 5HIAA has been our principle measurement of CNS serotonin functioning. This measurement has been criticized as lacking specificity. To assess the etiology of low CSF 5HIAA concentrations, we initiated studies using classic neuroanatomical techniques as well as nuclear medicine studies to assess neuroanatomical correlates of low CSF 5HIAA concentrations. Our collaboration with Drs. Stanley Watson and Juan Lopez (University of Michigan), to quantify neuroanatomical differences in serotonin receptors and mRNA and glucocorticoid receptors in monkeys with low or high CSF 5HIAA concentrations, was important in this regard. Fourteen monkeys were investigated. NMDA receptor *in situ* hybridization in prefrontal cortex and hippocampus was performed to assess 5HT1a and 5HT2a receptor mRNA. Analyses were also made to assess NR1, 2a, 2b, 2c and 2d mRNA as well as binding sites for glutamate, glycine, polyamines and pcp/mk801. Dopamine receptors (D1-D5) mRNA were assessed in the same regions. Glucocorticoid receptor mRNA and mineralocorticoid receptor mRNA were also assessed. Our initial results show large differences in the distribution of some of these molecules between rat and monkey, with the distribution of these molecules in monkeys resembling the distribution in humans more than rodents.

While neuroanatomical studies were being performed, we also initiated studies using PET, SPECT and NMR technology to longitudinally assess differences in monkeys with low and high CSF 5HIAA concentrations. PET studies were initiated to investigate corticotrophin releasing hormone differences in subjects with low and high CSF 5HIAA concentrations. Our SPECT studies continued to examine the role of differential serotonin receptors in subjects with low and high CSF 5HIAA concentrations. PET scans of monkeys with low and high CSF 5HIAA showed that when using a [C-11] alpha-methyl-L-tryptophan ligand, tryptophan uptake into the brain could be measured, but it bore no relationship to serotonin synthesis. NMR studies were initiated to investigate the role that early experiences play in normative CNS development.

RESEARCH HIGHLIGHTS: Our studies showed that in alcohol naive monkeys, objective measures of intoxication are positively correlated with a subjective rating for intoxication and, the degree of intoxication following a standardized alcohol dosage, is correlated with CNS serotonin functioning.

In a second project, our data showed that relative to baseline, concentrations of plasma cortisol and ACTH and CSF MHPG, HVA and 5HIAA (the major metabolites of norepinephrine, dopamine, and serotonin, respectively) were increased by a standardized IV alcohol infusion. Baseline 5HIAA, MHPG, HVA, ACTH and cortisol, as well as IV alcohol-induced MHPG, HVA and ACTH all predicted future voluntary alcohol consumption.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Offspring of alcoholics show a reduced pharmacological response to alcohol. Our findings suggest that a trait of low CNS serotonin turnover rate is conducive to increased "innate" tolerance to alcohol and a potential risk factor for excessive alcohol consumption

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00079-06 LCS

Title: PSYCHOBIOLOGY OF ANTISOCIAL BEHAVIOR AND HEALTH

Staff Years: 2.5

Principal Investigator: Higley JD, PhD (NN, LCS, NIAAA)

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Flory GS, BS (NN, LCS, NIAAA)
Goldstein MA, BS (NN, LCS, NIAAA)
Graham ST (NN, LCS, NIAAA)
Lindell S, BS (NN, LCS, NIAAA)
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Shoaf SE, PhD (UPS, LCS, NIAAA)
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Other Collaborators:
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Sample Type: Neither human subjects nor human tissues

Keywords: behavioral/social research, violence, neurosciences, sleep, sexually transmitted diseases, suicide, risk-taking behavior, gene mapping (nonhuman)

Summary: During the past year, our research included studies that investigated neurobiology and behaviors that are correlated with Type II-like excessive alcohol consumption, including aggression, impulsivity, sleep and circadian activity deficits and reduced affiliative social behavior. In an earlier study, we found that adolescent males with low CSF 5HIAA concentrations were more likely to exhibit violent, impulsive aggression and, as a consequence, to be killed in aggressive encounters. Subsequently we found that, even as adults, males with low CSF 5HIAA concentrations continued to die at greater rates than subjects with normal and high concentrations of CSF 5HIAA. As in our earlier study of adolescents, adult males with low CSF 5HIAA concentrations exhibited high rates of violent and escalated aggression but in these adults, this appeared to have adaptive consequences because high rates of escalated aggression resulted in high social dominance ranking. This later finding suggests that reduced serotonin turnover, as measured by low CSF 5HIAA concentrations in association with physical aggression, may be adaptive under certain conditions in some environments.

To assess the generalizability of our low CSF 5HIAA impulse deficit behavior findings, our rhesus macaques were compared to a closely related, but more peaceful and friendly macaque species (pigtail macaques). Between-species analyses indicated higher concentrations of CSF 5HIAA in pigtailed macaques as well as lower rates of high-intensity aggression, less unrestrained aggression and fewer

wounds. Within-species analyses indicated that interindividual differences in CSF 5HIAA concentrations were inversely correlated with escalated aggression and positively correlated with social dominance rank. These findings extend our previous studies and show that our earlier findings have generalizability (i.e., within both species, subjects that showed relatively high rates of excessive aggression were found to have low CSF 5HIAA concentrations).

Long-term Studies Comparing Rearing Conditions: Subjects were reared in one of three social conditions, in social groups with adults present (parent-reared) or in one of two social groups without adults (peer-only-reared, i.e., chronic peer-access) or (surrogate-peer-reared, i.e., daily exposure to age-matched peers but limited to only a few hours). All rearing conditions produced subjects exhibiting a full-range of species-typical behaviors when compared to both parent-deprived groups. Parent-reared subjects, however, were less likely to show aberrant behaviors. During the first six months of life, the surrogate-peer-reared subjects exhibited more advanced social behaviors relative to the peer-only-reared monkeys, the surrogate-peer-reared subjects showing higher levels of social play and less infant-like clinging. However, when all subjects were combined in a larger social group, beginning in the second year of life, surrogate-peer-reared subjects were more likely to exhibit aberrant behavior resulting in clinical intervention. The surrogate-peer-reared subjects also remained low in the social dominance hierarchy, never exceeding any of the peer-only- or parent-reared monkeys in social dominance ranking. On the other hand, over time, the peer-only-reared subjects equaled the parent-reared subjects in social dominance ranking.

Studies of how monkeys acquire social dominance showed, for the first time, that high-ranking monkeys exhibit accelerated physical development. Subjects that eventually became high and low ranking did not differing in size at the time of group formation but, after one year of being housed in the new social group, subjects acquiring a high social dominance rank showed increased size relative to their lower ranking cohort.

Maternal Behavior and Low CSF 5HIAA Concentrations: Previous research has shown that male macaques with low CSF 5HIAA concentrations exhibit high rates of aggression, wounds, social ostracism and impaired impulse control. Therefore, we hypothesized that infants of females with low CSF 5HIAA concentrations would exhibit less socially competent behaviors than those of females with high concentrations. During the past year, data were analyzed spanning a five-year period. Replicating data from previous research, mothers' and infants' CSF 5HIAA concentrations were positively correlated. Mother-infant dyads with low CSF 5HIAA levels spent significantly more time in ventral and social contact during weaning than did mother-infant dyads with high concentrations, suggesting a less smooth transition from maternal dependency to infant independence in dyads with low CSF 5HIAA concentrations. When compared to dyads with low CSF 5HIAA concentrations, infants of mothers with high CSF 5HIAA spent significantly more time in social interactions with other members of their group and more time independent. This also suggests a more difficult transition to independence and integration into macaque society for infants from dyads with low CSF 5HIAA concentrations. We concluded that, during the process of weaning, infants of mothers with low CSF 5HIAA concentrations exhibited less independent behaviors than infants of mothers with high CSF 5HIAA concentrations.

Child Abuse and Aggression in Females: In as much as studies from the laboratory show that females with low CSF 5HIAA concentrations exhibit high levels of physical violence, it would not be unreasonable to hypothesize that these females would be more likely to abuse or neglect their infants than the norm. To examine this hypothesis, we collaborated with Dr. Dario Maestripieri (Yerkes Regional Primate Research Center), who maintains a large population of female nonhuman primates that abuse and kill their offspring. Contrary to expectations, subjects who abused their infants exhibited high CSF 5HIAA concentrations. One explanation for this unexpected finding is that these females act less out of impulse deficits than high anxiety, similar to humans who exhibit post-traumatic stress disorder (PTSD). Further investigation continues.

In a recently completed study, we examined the relationship among impaired immune function, fearful behavior and left-handedness in female rhesus macaques. We used the percentage of CD4 (T-helper/inducer) and CD8 (T-suppressor/cytotoxic) cells to total T-cell lymphocytes and the CD4/CD8 ratio as dependent measures of immune function. We derived reactivity profiles from fearful and aggressive responses to an invasive threat and hand preference profiles from a quadruped food-reaching test. The results indicated negative correlation between left-handedness and CD4%, CD4/CD8 values and positive correlation between left-handedness and fearful behavior in response to an invasive threat. The results

of this study provide general support for the view that CNS function is associated with primate temperament and immune function and indicate that the degree of left-hand bias can be used as a behavioral marker to identify individuals at risk for immunosuppression and fearful temperament. Further analysis of handedness in nonhuman primates showed that adolescents with high cortisol and testosterone levels were more likely, as adults, to exhibit left handedness, suggesting a relationship between high cortisol and testosterone levels early in life and adult right hemisphere specialization.

RESEARCH HIGHLIGHTS: As in our earlier study of adolescents, the adult males with low CSF 5HIAA concentrations exhibited high rates of violent and escalated aggression but, in these adults, this appeared to have adaptive consequences, because high rates of escalated aggression resulted in high social dominance ranking. This later finding suggests that reduced serotonin turnover, as measured by low CSF 5HIAA concentrations in association with physical aggression, may be adaptive under certain conditions in some environments.

To assess the generalizability of our low CSF 5HIAA-impulse deficit behavior findings, our rhesus macaques were compared to a closely related but more peaceful and friendly species, the pigtail macaques. Between-species analyses indicated higher CSF 5HIAA concentrations in pigtailed macaques and lower rates of high-intensity aggression, less unrestrained aggression and fewer wounds. Within-species analyses indicated that interindividual differences in CSF 5HIAA concentrations were inversely correlated with escalated aggression and positively correlated with social dominance rank. These findings extend our previous findings and show that our earlier findings have generalizability, i.e., within both species individual subjects that show relatively high rates of excessive aggression also had low CSF 5HIAA concentrations.

Long-term studies comparing rearing conditions showed that during the first six months of life, the surrogate-peer reared subjects exhibited more advanced social behaviors relative to the peer-only reared monkeys. However, when all subjects were placed in a larger social group, beginning in the second year of life, the surrogate-peer-reared subjects were more likely to exhibit aberrant behavior resulting in clinical intervention. The surrogate-peer reared subjects also remained low in the social dominance hierarchy, never exceeding any of the peer-only- or parent-reared monkeys. On the other hand, over time, the peer-reared subjects equaled the parent-reared subjects in social dominance ranking.

Replicating data from previous research, mothers' and infants' CSF 5HIAA concentrations were positively correlated. We conclude that, during the process of weaning, infants of mothers with low CSF 5HIAA concentrations exhibit less independent behaviors than infants of mothers with high concentrations. Conversely, we found that females that abused their infants exhibited high CSF 5HIAA concentrations. One explanation may be that these females act less out of impulse deficits than high anxiety, similar to humans who exhibit post traumatic stress disorder.

The results of our study examining the relationship between impaired immune function, fearful behavior and left-handedness in female rhesus macaques indicated negative correlation between left-handedness and CD4% and CD4/CD8 values but positive correlation between left-handedness and fearful behavior in response to an invasive threat. These results provide general support for the view that CNS function is associated with primate temperament and immune function and indicate that the degree of left-hand bias can be used as a behavioral marker to identify individuals at risk for immunosuppression and fearful temperament.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Male type II alcoholics show a variety of behavioral deficits that may, in part, be a function of impaired CNS serotonin function. Much of our work focused on females with low CSF 5HIAA concentrations, showing that they exhibit behaviors that may be related to impaired impulse control that impacts on maternal behavior. Our work with other species suggests that these findings may have wide generalizability.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00081-06 LCS

Title: FUNCTIONAL MAGNETIC RESONANCE IMAGING OF EMOTION AS RELATED TO ALCOHOLISM

Staff Years: 5

Principal Investigator: Hommer DW, MD (BEI, LCS, NIAAA)

Other Personnel: Knutson BD, PhD (BEI, LCS, NIAAA)
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Rio DE, PhD (BEI, LCS, NIAAA)
Kaiser EA, BS (BEI, LCS, NIAAA)
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NIH Collaborators: Granger AJ (LCS, NIAAA)
Copeland CR, BS (LCS, NIAAA)
Parma-Dotson D (CN, LCS, NIAAA)
Rawlings RR, MS (CN, LCS, NIAAA)

Other Collaborators: None

Sample Type: Human subjects

Keywords: neurosciences, alcoholism, emotion, affect, MRI

Summary: Much of the recent work in the neurobiology of emotion has divided emotion into two categories, positive and negative emotion. Positive emotion is associated with approach behavior, while negative emotion is associated with avoidance. We have sought to characterize the neural substrates of approach and active avoidance behavior. Comparative studies have implicated a ventral forebrain dopaminergic path in approach behavior. We aimed primarily to verify activation of these areas in monetarily rewarded approach in humans and to determine if active avoidance behavior would elicit different patterns of activation. Twelve healthy right-handed males (age 20-40) participated in 10-minute approach and active avoidance tasks in counterbalanced order. During the approach task, subjects pressed a button in response to a target which followed either a reward cue (On condition) or a neutral cue (motor controlled Off condition), ISI ~ 5.5 s. If subjects responded before the reward target disappeared, they won $1.00, whereas their response to the neutral target did not affect their total. During the active avoidance task, subjects were given $20.00 and responded to targets that followed either a punishment cue or a neutral cue. If they failed to respond before the disappearance of the punishment target, they lost $1.00, whereas their response to the neutral target again did not affect their total. 200 T2*-weighted gradient echoplanar MR volumes depicting BOLD-contrast were acquired using a 1.5 Tesla GE Signa System. The volume consisted of 10 slices spanning the corpus callosum (voxel size; 3.8 x 3.8 x 7.0 mm, TR: 3000 ms). After correcting for in-plane motion, individual voxel activation was correlated with an ideal waveform corresponding to the expected activation timecourse using AFNI. The ideal waveform consisted of the task On-Off waveform convoluted with the hemodynamic response function. Significant voxels ($r > 0.30$, $p < 0.0001$) were highlighted on the functional images. For the approach task, significant activation was observed in the mesial prefrontal cortex, striatum, dorsomedial thalamus, right insula, and motor cortex. For the active avoidance task, a similar but more robust pattern of activation was observed. Relative to approach, active avoidance elicited increased volume of brain activity in all subjects. As predicted, monetarily rewarded approach-elicited activation in ventral medial areas (including the striatum and thalamus), as well as more dorsal medial regions (i.e., mesial prefrontal cortex). Monetarily punished active avoidance appeared to elicit even greater activation in these areas, as well as cingulate areas. These data provide the first neural corroboration of the psychological phenomenon of loss aversion, since subjects had a "greater" neural response to loss than to an equivalent expected gain. In a second experiment, we sought to distinguish brain activity associated with anticipation of incentives from brain activity associated with responding for incentives. Analysis of fMRI

data collected from 8 females indicated activation of more rostral parts of this circuitry during anticipation of incentives (i.e., caudate, putamen, mesial prefrontal cortex) versus more caudal aspects of this circuitry during response for incentives (i.e., thalamus, supplementary motor area, premotor area). These results suggest that rostral midline paralimbic circuitry plays a prominent role in the anticipation of reward and punishment.

RESEARCH HIGHLIGHTS: These data provide the first neural corroboration of the psychological phenomenon of loss aversion, since subjects had a "greater" neural response to loss than to an equivalent expected gain. In a second experiment, we sought to distinguish brain activity associated with anticipation of incentives from brain activity associated with responding for incentives. Analysis of fMRI data collected from 8 females indicated activation of more rostral parts of this circuitry during anticipation of incentives (i.e., caudate, putamen, mesial prefrontal cortex) versus more caudal aspects of this circuitry during response for incentives (i.e., thalamus, supplementary motor area, premotor area). These results suggest that rostral midline paralimbic circuitry plays a prominent role in the anticipation of reward and punishment.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The study of the neural basis of reward and punishment is critical for understanding the neural basis of alcoholism and drug addiction. These studies will allow us to determine if there are differences in brain organization and response as regards reward and punishment between alcoholics and non-alcoholics.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00082-06 LCS

Title: STATISTICAL ANALYSIS OF IMAGE FEATURES

Staff Years: 1

Principal Investigator: Rio DE, PhD (BEI, LCS, NIAAA)

Other Personnel: Knutson BD, PhD (BEI, LCS, NIAAA)
Hommer DW, MD (BEI, LCS, NIAAA)
Shen J, MD (BEI, LCS, NIAAA)
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NIH Collaborators: Rawlings RR, MS (CN, LCS, NIAAA)

Other Collaborators: Momenan R, PhD (MedData)

Sample Type: Human Subjects

Keywords: neurosciences, imaging, PET, MRI

Summary: The aim of this project is the development of statistical methods that either take into account interpixel correlation or apply global image transform methods that permit an analysis of uncorrelated image components. Of typical interest is the investigation of differences between images from individual subjects acquired under different experimental conditions or between average images of subjects from different diagnostic groups. Three different statistical methods have been developed, based on the Fourier transform, the wavelet transform, and the theory of Gaussian random fields in the spatial domain. In the Fourier domain, the statistics at different wave numbers are uncorrelated and inference tests can be performed unencumbered by spatial correlations. This method provides for rigorous statistical tests with well-known properties and interpretations, but results in spatially uniform image blurring and may yield relatively poor spatial localization. For the wavelet-transform-based analysis, a mathematically rigorous theory has been established that applies parametric statistical tests on wavelet coefficients and results in estimates of local image differences by inverse wavelet transform of only significant coefficients. The method provides for good spatial localization and the implementation of locally adaptive image smoothing, but there has not been much experience accumulated for the interpretation of test outcomes and estimates of image differences. Gaussian random field analysis has good spatial localization properties and permits the investigation of correlations with external variables (e.g., age), but it results in spatially uniform image blurring and does not provide for statistically reconstructed estimates of images differences (either across group or conditions). All three methods have been applied to the analysis of PET images from normal and alcoholic subjects and have identified significant differences in generally the same brain regions. Gaussian random field analysis was able to demonstrate, in PET images from alcoholics, a significant negative correlation of glucose utilization in the pre-frontal cortex with age. Current research on these topics includes the development of a 1-D Gaussian random field method to analyze fMRI time series data. This methodology can be used to analyze fMRI data acquired from experiments designed to incorporate a long (that is long enough, as determined experimentally, to estimate the variance associated with the acquired data) baseline condition and transition to another activated state. It uses the long baseline data to estimate the variance measure associated with the temporal data from a voxel within the image and sets a statistically rigorous threshold for activation in spite of the known temporal correlation in the data. This analysis technique is being validated with simulated and experimental data. Furthermore, this analysis technique is being incorporated into numerous experiments including one designed to look at the blood flow changes in the brain associated with alcohol intake in normal subjects. This presents an ideal demonstration of this analysis technique to basically establish a response curve for alcohol intake. Finally, statistical analysis in the temporal domain, based on traditional time series analysis in the Fourier domain, have been developed and give similar results in terms of localization of the signal in fMRI blood flow studies to other less rigorous and generalizable techniques. This analysis methodology has the potential to: (1) localize fMRI activation changes, (2) estimate or reconstruct the activated signal without the associated noise, (3) estimate the hemodynamic response function locally without prior assumptions as to its structure, and (4)

detect multiple responses to multiple input stimuli. Currently this technique is being used to study both simple finger tap data as well as more complex experimental designs.

RESEARCH HIGHLIGHTS: Applications of the 2/3-D Gaussian random field theory in the spatial domain have continued to yield results in the Section's main research area of alcoholism and associated areas of smell and head injury. The initial application of the 1-D Gaussian random field theory in time has shown promise and an abstract will soon be submitted for presentation of preliminary results. Preliminary results from the Fourier domain analysis of time series data has been presented at the 5th International Conference on Functional Mapping of the Human Brain (HBM99): Statistical analysis of functional magnetic resonance images in the Fourier domain using a linear time invariant model.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The analyses methods developed have applications in any field of biomedical research involving imaging. They are particularly important in the field of alcoholism research studying the effects of alcohol on the brain or in studies showing links between a predisposition to alcoholism that may be associated to prior brain functions.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00092-04 LCS

Title: MONITORING OF HEART RATE VARIABILITY DURING ALCOHOL WITHDRAWAL SYNDROME

Staff Years: 0.8

Principal Investigator: DePetrillo PB, MD (UCBP, LCS, NIAAA)

Other Personnel: Karimullah K (UCBP, LCS, NIAAA)

NIH Collaborators: Hommer DW, MD (BEI, LCS, NIAAA)

Other Collaborators: None

Sample Type: Human subjects

Keywords: heart, alcohol withdrawal, ethnicity, gender, signal dynamics

Summary: The purpose of the project was to examine measures of heart rate variability (HRV), continuously and noninvasively, in male and female alcoholics admitted for drinking cessation. The peripheral manifestations of alcohol withdrawal syndrome (AWS) are characterized by rapidly changing autonomic influences. These may vary, minute to minute and hour to hour, depending on the severity and stage of AWS. We developed measures of HRV which are relatively insensitive to non-stationarity of signal and capture relevant data during short periods of measurement. We are now analyzing data from human subjects admitted for treatment of alcohol withdrawal syndrome. Alcoholic subjects are known to have IBI time-series which are significantly less complex than healthy comparison subjects. Our question was whether abstention from alcohol use for a period of 4 to 6 weeks would result in normalization of the complexity measure back to baseline, as defined by healthy comparison subjects, or if the decreased signal complexity remains stable for a period of 4 to 6 weeks. Preliminary analysis of this data shows that cardiac signal complexity increases with the length of time since the last intake of alcohol. However, even at 4 weeks, the measure does not return to the expected baseline. This suggests that: a) the length of time required for the measure to return to baseline is longer than 4 weeks; b) chronic excessive alcohol intake results in permanent autonomic disruption resulting in altered cardiac signal dynamics; or, c) subjects who consume excessive amounts of alcohol have a premorbid alteration in autonomic function. Future work related to this project will address possible mechanisms of alterations in cardiac signal complexity. A major question to be answered is whether the alterations in cardiac signal dynamics we have found are associated with sub-clinical abnormalities of cardiac function.

RESEARCH HIGHLIGHTS: Developed a sensitive method to measure the regulation of heart rate by the nervous system. Determined that chronic excessive alcohol use alters the timing of heartbeats. Showed that the change in this timing measure tends to return back to normal after 3 - 4 weeks of abstinence. When measured 3 - 4 weeks after the initiation of abstinence, the timing of heartbeats are still abnormal in many subjects

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Chronic excessive alcohol use alters the timing of heartbeats; such alteration in other diseases correlated with sudden death from abnormal heartbeats. The findings suggest that chronic excessive alcohol use may be associated with deleterious effects on heart function, which may or may not be reversible with abstinence. The findings also suggest that subjects who have pre-existing abnormalities with the regulation of heartbeat timing may be more prone to disruption of the timing of heartbeats induced by alcohol. Therefore, this should be taken into account before moderate alcohol use is encouraged as a way to decrease heart disease.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00093-04 LCS

Title: INTERACTION OF ETHYL ALCOHOL WITH CELLULAR CYSTEINE PROTEASES

Staff Years: 1.4

Principal Investigator: DePetrillo PB, MD (UCBP, LCS, NIAAA)

Other Personnel: Li X, PhD (UCBP, LCS, NIAAA)
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NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: toxicology, neurosciences, molecular genetics

Summary: Certain types of cellular cysteine proteases may play an important role in modulating the activity of G protein-coupled receptors and the activity of this class of enzymes is closely regulated by cytoplasmic and nuclear inhibitors. The interaction of these inhibitors with the target proteases is, in part, mediated by strong hydrophobic interactions. The effects of ethyl alcohol exposure on cysteine protease activity have been studied in cell culture utilizing a PC12 cell line. Activity of these proteases appears to be strongly affected by alcohol exposure. We previously established that exposure of PC12 cells to ethyl alcohol, for 96 hours, results in a decrease in calcium-stimulated protease activity. We are working towards defining a mechanism for this alcohol-induced inhibition of calcium-activated protease activity. We have preliminary evidence that calpain may be redistributed in PC12 cells as a result of ethanol exposure, and that this redistribution alters the ability of detergents to extract calpain protein from cells. We have also found that while levels of calpastatin, the endogenous inhibitor of calpain, are not significantly altered after ethanol exposure there may be differences in its ability to inhibit calpain based on post-translational modifications. One possibility is that the phosphorylation of serine residues in the protein is altered after ethanol exposure. We are in the process of determining the extent and significance of these alterations in modulating the inhibitory activity of calpastatin after ethanol exposure.

RESEARCH HIGHLIGHTS: Alcohol exposure alters the function of cell proteins called calpains, which are also called cysteine proteases. Calpains are involved in modulating the messages between nerve cells; when abnormally activated, they are associated with cell death. Abnormal function of calpains may be associated with alcohol-related nerve cell death.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Alcohol-related toxicity on brain cells and brain function may be related to abnormal calpain function after alcohol exposure. It may be possible to decrease the toxic effects of alcohol on brain function if the mechanisms associated with toxicity are discovered.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00094-04 LCS

Title: YOHIMBINE CHALLENGE TO STUDY NORADRENERGIC FUNCTION IN INDIVIDUALS

Staff Years: 7.1

Principal Investigator: George DT, MD (CS, LCS, NIAAA)

Other Personnel: Umhau JC, MD (CS, LCS, NIAAA)
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Biddison JR, BA (CS, LCS, NIAAA)
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NIH Collaborators: Hill DM, SW (CABC, LCS, NIAAA)

Other Collaborators: None

Sample Type: Interview, questionnaires, or surveys only

Keywords: behavioral research, violence, neurosciences, spouse abuse

Summary: Domestic violence is a major problem affecting society. Results from our research show that individuals who are physically abusive to their significant other frequently experience a hyperaroused state at the time of the violent act which is characterized by increased motor activity, heightened autonomic nervous system activity and accompanied by feelings of "being out of control". This hyperaroused state is similar to that which occurs in Post-Traumatic Stress Disorder (PTSD). Since PTSD has been associated with changes in noradrenergic function, we postulate that subjects who lose control and are physically violent may also have abnormal noradrenergic function. To test this hypothesis, we have designed a study which compares the behavioral and biochemical effects resulting from the administration of the alpha-2 antagonist, yohimbine, between a group of patients who lose control and are physically violent and non-violent comparison subjects. To date there have been no untoward effects, but subject accrual has been temporarily suspended pending analysis of preliminary data.

RESEARCH HIGHLIGHTS: Our preliminary results suggest that yohimbine does not cause the marked behavioral change in perpetrators that it does in subjects with panic disorder or PTSD. Although perpetrators are more likely to report aggressive feelings, the lack of a pronounced behavioral effect following yohimbine argues against the presence of increased noradrenergic activity in alcoholic perpetrators of domestic violence. Laboratory analysis for plasma MHPG and NE is pending.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: These results do not support the theory that perpetrators of domestic violence have changes in their noradrenergic system as a result of previous exposure to domestic violence growing up.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00095-04 LCS

Title: ISOLATION/CHARACTERIZATION OF SULFONYLUREA-LIKE COMPOUNDS AND INSULIN RELEASE

Staff Years: 0.1

Principal Investigator: Eskay R, PhD (NN, LCS, NIAAA)

Other Personnel: None

NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: behavioral/mental health research, toxicology, neurosciences, endocrine system, CNS, methodological studies

Summary: Selected serotonergic compounds, through a CNS-site of action, appear to be able to release sulfonylurea-like peptides from the pituitary gland. The therapeutic significance of sulfonylurea compounds, like glibenclamide and tolbutamide, in treating type II diabetes by regulating plasma glucose levels has been recognized for decades without a full appreciation of the mechanism of action of these compounds, nature of the receptor or demonstration of the existence of endogenous ligands. After extensive isolation and characterization of sulfonylurea-like activity in the CNS and pituitary gland, several peptide fractions have emerged from the pituitary gland which: (1) enhance insulin secretion, (2) inhibit sulfonylurea drug binding to sulfonylurea receptors, and (3) appear to mimic the electrophysiological membrane changes observed with sulfonylurea drugs. A novel peptide and a known peptide with a novel function have emerged from these studies. It is envisioned that there exists a family of novel endogenous peptides that modulate sulfonylurea receptors. We have demonstrated that a member of the non-histone nuclear protein group mimics many of the actions of the sulfonylurea compounds and this compound appears to be an intracellular regulator of the sulfonylurea receptor. Only data analysis and manuscript work was performed this fiscal year.

RESEARCH HIGHLIGHTS: The goal of this project was to isolate and characterize endogenous compounds that could mimic the ability of the pharmacological sulfonylurea compounds, widely used in treatment of type II diabetes, to enhance insulin release and glucose disposal. In the process, a known peptide consisting of 89 amino acids was discovered and shown to be a regulator of the ATP-dependent potassium channel and to release insulin.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Sulfonylurea compounds, such as tolbutamide and glibenclamide, are widely used in the clinical management of noninsulin-dependent diabetes mellitus (NIDDM). Further characterization of the intrinsic compounds that mimic the action of the sulfonylurea compounds should contribute to a better understanding of pancreatic hormone secretion and NIDDM.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00097-04 LCS

Title: COMPARATIVE ETHANOL METABOLISM IN NORMAL VOLUNTEERS

Staff Years: 1

Principal Investigator: Shoaf SE, PhD (UPS, LCS, NIAAA)

Other Personnel: Jie W, MD (UPS, LCS, NIAAA)
Singley ED, BS (UPS, LCS, NIAAA)

NIH Collaborators: George DT, MD (CS, LCS, NIAAA)
DePetrillo PB, MD (UCBP, LCS, NIAAA)
Lionetti T, BSN, MA (CC)

Other Collaborators: None

Sample Type: Human tissues, fluids, cells, etc.

Keywords: alcohol dehydrogenase, men, women, genotype, pharmacokinetics, CYP2E1, Hispanic, Asian, Caucasian

Summary: The incidence of alcohol-related disease is different in different populations. The allelic frequency of the enzymes thought to be primarily responsible for alcohol metabolism is also different in these populations. Alcohol distributes in total body water, therefore, doses of alcohol should be made on a g/l of total body water basis instead of a g/kg basis. Previous attempts to compare ethanol metabolism in different ethnic groups did not compare individuals of similar genotype or adjust the dose to account for possible differences in total body water. The rate of ethanol metabolism may be dependent on the NADH/NAD ratio. Markers of the NADH/NAD ratio, beta-hydroxybutyrate/acetoacetate (B/A) and lactate/pyruvate (L/P), were found to increase very quickly following ethanol consumption. The ratio remained at a plateau while ethanol concentrations declined according to zero-order kinetics. The B/A and P/L ratios fall rapidly just before ethanol concentrations decline according to first-order kinetics.

RESEARCH HIGHLIGHTS: Insufficient subjects enrolled to allow data analysis.

SIGNIFICANCE TO BIOMED RESEARCH & PROGRAM: The incidence of alcohol-related diseases is different in different ethnic groups. This study is designed to determine if the rate of ethanol metabolism is different in different ethnic groups and, if so, is this difference due to the different forms of the enzymes that are responsible for the metabolism of ethanol.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00098-04 LCS

Title: DISTRIBUTION OF SEROTONERGIC NEURONS USING (11-C) COMPOUNDS AND PET IMAGING

Staff Years: 0.2

Principal Investigator: Shoaf SE, PhD (UPS, LCS, NIAAA)

Other Personnel: None

NIH Collaborators: Carson R, PhD (PETD, CC)
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Herscovitch P, MD (PETD, CC)
Hurley AC, BS (NN, LCS, NIAAA)
Pushkas JG, BS (NN, LCS, NIAAA)
Baldwin GP, BS (CC)
Thomas III ML, DVM (SRU, SSB, OD)

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: serotonin, brain, rhesus monkey, alpha-methyl-tryptophan, antagonists, 5HT1A, 5HT2A, PET

Summary: Our first study indicated that serotonin synthesis rates determined by the use of alpha-methyltryptophan (MTP) and PET imaging were found to be highly correlated to free plasma tryptophan concentrations, a parameter that is not well correlated to the regulation of serotonin synthesis rates under steady state conditions, and not to cerebrospinal fluid concentrations of 5-hydroxy-indole-3-acetic acid, long considered a measure of central serotonin turnover (Neuropsychopharmacology 1998;19(5):345-53). In order to determine what is actually being measured by alpha-MTP uptake, 11-C and 14-C labeled compound were co-administered to four rhesus monkeys. After a one-hour Positron Emission Tomography (PET) scan, animals were euthanized and brain tissues were taken and frozen at -80 degrees C. HPLC analysis with fluorimetric detection and fraction collection indicates that alpha-MTP is minimally converted to alpha-methylserotonin; there is less than 4% conversion in the dorsal raphe nucleus and less than 0.6% in cortical regions. We conclude that alpha-MTP is acting primarily as a tracer of tryptophan uptake in the one hour following intravenous infusion and is unsuitable for measuring serotonin synthesis rate using PET (Journal of Cerebral Blood Flow and Metabolism, in press). Development of ligands for detection of serotonin receptors continues. A study is currently being performed with [11-C] MDL-100907. A protocol is being written for use of a fluorine derivative of WAY-100635.

RESEARCH HIGHLIGHTS: [11-C] Methyl-L-tryptophan was shown to not act as a tracer of serotonin synthesis but as a tracer for tryptophan uptake.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Other investigators who have used this ligand and interpreted their results assuming [11-C] MTP is a marker of serotonin synthesis will have to re-evaluate their data. Currently more than a dozen papers will have to be re-reviewed. Our institute is suspending its investigations with [11-C] MTP.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00120-02 LCS

Title: ETHANOL PHARMACOKINETICS FOLLOWING INTRAVENOUS INFUSION TO HUMANS

Staff Years: 1

Principal Investigator: Shoaf SE, PhD (UPS, LCS, NIAAA)

Other Personnel: Jie W, MD (UPS, LCS, NIAAA)

NIH Collaborators: DePetrillo PB, MD (UCBP, LCS, NIAAA)
George DT, MD (CS, LCS, NIAAA)
Lionetti T, BSN, MA (CC)

Other Collaborators: None

Sample Type: Human tissues, fluids, cells, etc.

Keywords: pharmacokinetics, alcohol, men, women, modeling, beta-hydroxybutyrate, acetoacetate

Summary: Ideally a pharmacokinetic model determined following one dose of ethanol should be able to predict blood concentrations following administration of a different dose or a dose infused over a longer or shorter period of time. Previously constructed models developed for men do not do this. In this project, a dose of ethanol will be administered to the same individual over three different time periods on three separate days. Both men and women will be studied. Pharmacokinetic analysis will be performed to determine the suitability of a two-compartment, open model with dual elimination pathways to fit blood alcohol concentrations obtained following all three times of infusion. Initial pharmacokinetic analysis reveals that distribution of ethanol is saturable. Following rapid infusions, blood alcohol concentrations drop 30% within 15 min following the cessation of the infusion; there is no similar drop when the infusion rate is slower. The size of the smaller compartment is approximately 20% of total body water. Peak blood alcohol concentration is very dependent on a subject's total body water. When ethanol is dosed as g/L total body water, peak concentrations are similar in men and women. Dual elimination pathways are necessary to adequately model concentration 15-45 min post-infusion. A single elimination pathway can adequately predict terminal concentrations with loss of fit at the early post-infusion times. Elimination and distribution parameters are similar in men and women.

RESEARCH HIGHLIGHTS: In order to ensure that all individuals are exposed to the same concentrations of alcohol, you must dose according to liters of total body water, not body weight. Distribution of ethanol is confined to the body water.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Pharmacokinetic models of alcohol have been limited by the lack of computing power and data sets with enough values to provide adequate characterization of the absorption and elimination profiles. Currently, there is no way to estimate a blood alcohol curve prior to its administration. No data has been obtained from women. This will provide adequate data sets for modeling and will allow us to determine if the model is the same for men and women.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00121-01 LCS

Title: ROLE OF CYP2E1 mRNA IN INDUCTION OF CHLORZOXAZONE HYDROXYLASE ACTIVITY

Staff Years: 0.8

Principal Investigator: Shoaf SE, PhD (UPS, LCS, NIAAA)

Other Personnel: Jie W, MD (UPS, LCS, NIAAA)

NIH Collaborators: George DT, MD (CS, LCS, NIAAA)
Horne MK, MD (CC)
Umhau JC, MD (CS, LCS, NIAAA)
Herion DW, MD (LD, DDB, NIDDK)
Lionetti T, BSN, MA (CC)
Liang TJ, MD (LD, DDB, NIDDK)

Other Collaborators: None

Sample Type: Human tissues, fluids, cells, etc.

Keywords: alcoholic, human, CYP2E1, protein, mRNA, lymphocyte, ketosis, liver, biopsy, chlorzoxazone

Summary: CYP2E1 is a cytochrome P450 enzyme thought to be involved in liver toxicity following ethanol (alcohol) consumption. Chlorzoxazone (CZ) is a drug that, *in vivo*, is metabolized primarily (>95%) by CYP2E1. Several groups have proposed using the rate of CZ metabolism as a marker of CYP2E1 concentrations. During heavy alcohol consumption in rats, CYP2E1 protein concentrations are elevated. In recent studies, it was expected that all alcoholics withdrawn for 24 hr would have elevated rates of CZ metabolism, but it was found that approximately 20% have CZ metabolism rates in the range of normals. The investigators concluded that some individuals must have "noninducible" CYP2E1. We believe that their result was due to the fact that, following alcohol administration to rats, CYP2E1 concentrations have been shown to be elevated in the following ways: 1) The presence of alcohol stabilizes the CYP2E1 enzyme and prevents its degradation, increasing the turnover time and increasing CYP2E1 concentrations. When alcohol is eliminated, CYP2E1 is rapidly degraded (half-life of <6 hours) and concentrations return to control values. 2) CYP2E1 mRNA concentrations are increased, which results in more CYP2E1 production. Disease states or feeding regimens, with or without alcohol, which produce ketoacidosis induce CYP2E1 through the second mechanism. In this case when alcohol is eliminated, CYP2E1 concentrations remain high and the loss of CYP2E1 is dependent on the rate of loss of CYP2E1 mRNA concentrations. Thus, we believe that alcoholics with elevated CZ metabolism had elevated CYP2E1 mRNA concentrations and alcoholics with normal CZ metabolism did not. We will measure CYP2E1 protein and mRNA lymphocyte concentrations (as a marker of liver concentrations). As CZ metabolism might be affected by liver disease, a liver biopsy will be performed to make a definitive diagnosis; extra tissue from the biopsy will be used to determine CYP2E1 protein concentrations. Because elevations of CYP2E1 mRNA have been associated with ketonemia, we will also investigate whether alcoholics have ketonemia at admission or may have had ketonemia very recently. At admission, acetone, beta-hydroxybutyrate, acetoacetate, Hemoglobin A1c, insulin and C-peptide concentrations in blood will be measured. Subjects will also be asked to complete a food diary for the week preceding admission, since behavior such as not eating for 24 h will produce ketonemia. A protocol has been approved.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00212-02 LCS

Title: ORGANOTYPIC CULTURE OF ETHANOL SENSITIVE REGIONS OF THE CNS: NEUROTOXICITY MODEL

Staff Years: 1.57

Principal Investigator: Eskay R, PhD (NN, LCS, NIAAA)

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NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: organotypic culture, explants, neurotoxicity, brain, hippocampus, entorhinal cortex, neurodegeneration, oxidative stress

Summary: A series of whole animal studies have clearly demonstrated selective brain neurodegeneration in a binge-type alcohol rodent model. Pyramidal cells of layer III of the entorhinal cortex (ENT) and hippocampal (HIPP) dentate gyrus granule cells are particularly vulnerable to the cytotoxic consequences of binge-type alcohol exposure. The main purpose of this project was to establish an experimental model to investigate alcohol-induced neurodegeneration *in vitro* that would complement our *in vivo* studies. Organotypic rat brain slices provide a culture system that preserves the main morphological and neurochemical features of brain tissue. Furthermore, organotypic slice cultures retain their native circuitry, cell types and synaptic density with conservation of main afferents. Ongoing studies have revealed that the ENT-HIPP explants are optimal for study at 2-3 weeks post harvest when slices are obtained from 8-day old neonates. The ENT-HIPP explants were treated with ethanol (0.2-0.6%) over 6 days using several different alcohol exposure paradigms. Alcohol-induced neuronal injury and/or death were assessed by the extent of uptake of propidium iodide (PI, cytotoxic marker) and the amount of lactate dehydrogenase (LDH), another cytotoxic marker, in the explant medium. Treatment of ENT-HIPP slices cultured in serum-free media containing B27 neuronal supplement with 0.2-0.4% alcohol for 2-6 days resulted in dose- and time-related increases in PI uptake and LDH leakage. The presence of classical antioxidants (glutathione, vitamin E, catalase and superoxide dismutase) did not lessen the alcohol-induced cytotoxicity. Furthermore, agents that were found to be cytoprotective *in vivo* in the presence of alcohol, such as furosemide and cannabadiol, were not protective *in vitro*. The study of alcohol's effects on brain explants of the HIPP-ENT area, as an adjunct to *in vivo* studies, appears not to be a reliable predictor of alcohol-induced neurodegeneration or a system to evaluate possible therapeutic cytoprotective agents.

RESEARCH HIGHLIGHTS: Organotypic cultures of brain regions sensitive to ethanol exposure have revealed that dose and duration of exposure have endangering or cytotoxic effects on the brain explants. Initial studies indicate that the addition of antioxidants to the cultures lessen the cytotoxicity of ethanol exposure.

SIGNIFICANCE TO BIOMED RESEARCH & PROGRAM: Organotypic cultures of selected brain regions with intact neuronal circuitry and support cells should provide a more physiological and, therefore, more informative model system than homogeneous, isolated cell cultures. The ease of testing potential cytoprotective compounds against alcohol toxicity should hasten the development of therapeutic cytoprotectants.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00226-02 LCS

Title: MODULATION OF CALPASTATIN-CALPAIN INTERACTIONS BY ETHANOL

Staff Years: 1.8

Principal Investigator: DePetrillo PB, MD (UCBP, LCS, NIAAA)

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NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: alcoholism, neurotoxicity, PC12, post-translational modifications, protein-protein interactions, calpain, calpastatin, proteases

Summary: Exposure of PC12 cells to ethanol results in large changes in calcium-stimulated protease activities. Since these proteases are critical modulators of both neurotransmitter release as well as cellular toxicity and death, we are exploring the hypothesis that ethanol-mediated neural toxicity may result from calcium-activated protease dysregulation. Calpastatin, a calpain inhibitor, is an acidic, hydrophobic protein that interacts with the hydrophobic active site(s) of mu- and m-calpains. A series of post-translational modifications of calpastatin have been described that alter the binding affinity to calpains, among them, PKC-mediated phosphorylations. Using a PC12 model, we are examining the effects of ethanol exposure and withdrawal on protein-protein interactions. Because of the hydrophobic nature of calpastatin-calpain interactions, we examined the possibility that ethanol might modify protease-calpastatin (inhibitor) complex stability. We found that exposure of PC12 cells to ethanol results in an increase in the molecular weight of calpain and calpastatin-containing protein complexes, and that this is associated with a change in protease activity. We are extending these observations by use of immunocytochemical techniques, where the nature of these complexes and their cellular locations will be examined.

RESEARCH HIGHLIGHTS: We have identified a process by which certain proteins within cells may clump together in the presence of alcohol, thereby altering their function. These proteins are called calpains, and they can cause cell death if they are inappropriately activated. We believe that one of the proteins involved is calpastatin, an inhibitor of the calpains.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Identification of one of the mechanisms of alcohol toxicity may provide a framework for pursuing strategies which decrease toxicity. Alteration of protein-protein interactions by alcohol may also provide answers to other cell related effects of alcohol.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00277-11 LCS

Title: MODELS OF CNS SEROTONIN FUNCTIONING: ALCOHOL CONSUMPTION AND IMPAIRED IMPULSES

Staff Years: 2.5

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Sample Type: Neither human subjects nor human tissues

Keywords: behavioral/social research, violence, neurosciences, sleep, sexually transmitted diseases, suicide, risk-taking behavior, gene mapping (nonhuman)

Summary: One of the most powerful advantages of the primate model, developed by our laboratory, is the capacity to closely control and investigate the role of genes and environment using a developmental paradigm. During the past year, our research included studies of genetic and environmental mechanisms that produce low cerebrospinal fluid (CSF) concentrations of

5-hydroxyindoleacetic acid (5HIAA) and alcohol consumption. We continued our pedigree analyses investigating genetic and environmental influences on alcohol consumption. In an analysis of factors affecting alcohol consumption in nonhuman primates, that for the first time utilized a large population sample, we were able to show that age, sex, drinking conditions, rearing background and strain were important influencing variables. When these where controlled, heritable influence contributions accounted for about 30% of the variance in alcohol consumption in macaques. In the same study, we replicated earlier findings showing that monkeys reared in peer-only groups, without adults present, were more likely to consume alcohol in excess.

Molecular genetic studies were also pursued. In collaboration with Drs. K Peter Lesch and Armin Heils (University of Wurzberg, Germany), we found an association between length variation in the serotonin transporter gene regulatory region (5HTTLPR), CSF 5HIAA concentrations and competitive aggression. In humans, 5HTTLPR is associated with anxiety, affective disorders and substance abuse, but not consistently. The principle finding reported, a genotype/environment interaction effect on CSF 5HIAA, suggests a potential source to explain serotonin variability found in the human literature. Data showed a similar effect on aggression, with monkeys having the short allele exhibiting more aggression. Unlike the effect on CSF 5HIAA concentrations, this augmenting effect on aggression appeared to be independent of rearing condition. This developmental model was also used to assess the developmental pathways for differences in behavior and CSF 5HIAA concentrations. In collaboration with Drs. Maribeth Champoux and Stephen Suomi (NICHD), we assessed the contribution of genetic and environmental variables on development during a standardized neonatal examination of macaque infants. Assessments were conducted on 322 laboratory-born infants on postnatal days 14 and 30. Test items were condensed into Orientation, Motor Maturity, Activity and State Control clusters (measures of motoric development, cognition and temperament). Heritability values significantly greater than zero ($p < 0.05$) were obtained for State Control and Orientation clusters on days 14 and 30, for the Activity cluster only on day 14, but not for Motor Maturity. Heritability values changed with age, with the pattern of change differing across clusters. In a second study with this group, we found that neonatal monkeys with the short allele were deficient in the neonatal measures designed to assess CNS maturity and development. Other data show that more aggressive strains, such as the Chinese rhesus are more likely to possess the less efficient serotonin transporter genotype (5HTTLPR-short allele) first characterized by Lesch. These findings suggest low CSF 5HIAA concentration results, at least in part, from an altered gene expression in the transporter region, but that early rearing experiences affect its phenotypic expression. This may result in behavior differences that can be tracked developmentally.

Three other projects involving analysis of other candidate serotonergic genes are underway. In a collaboration with Drs. David Goldman (LNG) and Andrew Bergen (NCI), monkeys have been typed for a MAO-A polymorphism similar to one associated with panic disorders in humans. By analyzing monkeys' acute response to a social stressor, we found preliminary evidence for an association with the MAO-A genotype and stress reactivity. In a second project, the serotonin 1A receptor was assessed as a target for candidate gene analysis and a large set of monkeys genotyped for a 5HT1A polymorphism. Analyses are underway to assess the phenotypic expression of the discovered polymorphisms. THROMBOCYTE-MAO (trbcMAO) is, in part, genetically determined and is stable throughout life. There are a number of studies showing a correlation between trbcMAO and personality traits such as sensation seeking and impulsiveness, which in turn underlies connections between low trbcMAO and vulnerability for substance abuse and violent behavior. A third project involved a collaboration with Drs. Marie Asberg and Lars Oreland and led to finding a correlation between trbcMAO and CSF 5HIAA in nonhuman primates. Moreover, we found a positive correlation between trbcMAO, impaired social dominance and excessive alcohol intake. These findings suggest that trbcMAO may act as a biological marker for central serotonin system function, which is related to social functioning and excessive alcohol consumption.

A computerized apparatus was developed that enabled us to identify individual monkeys living in large social groups, dispense alcohol to them and measure individual consumption patterns, thus allowing us to characterize large numbers of monkeys as high and low alcohol consumers. As recommended by our Scientific Counselors, the apparatus was modified to provide a clearer choice between the sweetened alcohol solution and the sweetened vehicle that the alcohol solution is made from. This methodology will allow us to study chronic alcohol consumption 24-hours a day, for the first time, and provide a technical solution to allow other researchers interested in modeling alcohol consumption in primates to perform such studies using limited human resources.

A growing body of evidence indicates that corticotrophin releasing hormone (CRH) plays an important role in many of the classic behavioral and physiological responses to stress, including anxiety, activation of the hypothalamic-pituitary adrenal (HPA) axis and stimulation of the sympathetic and adrenomedullary systems. This is important to alcohol research because stress and anxiety are thought to play a primary role in excessive alcohol consumption. In a new initiative, we administered a non-peptide CRH type 1 receptor antagonist, antalarmin, to monkeys under stress. This collaboration with Drs. Phil Gold and Kamal Habib (NIMH) showed that antalarmin significantly reduced indices of anxiety while enhancing environmental exploration, a behavior that typically occurs in the context of a non-threatening environment. In addition, antalarmin significantly inhibited stress-induced activation of the HPA axis, sympathomedullary system and stress-induced increases in CSF CRH. These data demonstrate a significant role for CRH in the regulation of the stress system in nonhuman primates and suggest that CRH antagonists may be helpful in treating human disorders associated with stress system dysregulation such as excessive alcohol consumption, anxiety disorders and depression. Early life parental deprivation has been shown to predispose monkeys to major depression, excessive alcohol consumption and anxiety disorders later in adulthood. In a second series of studies, we separated six-month old rhesus monkeys (N=17) from their mothers for a period of four weeks. CSF samples from those seventeen monkeys were obtained before and, weekly, after separation. We found a significant correlation between the time after separation and CSF levels of CRH (Pearson $r = 0.4814$, $p < 0.0001$), suggesting that social stress induces a vigorous CRH response in infant monkeys.

Our new program of study, designed to investigate cognitive function and impulsivity in monkeys prone to high alcohol consumption and/or low CSF 5HIAA concentrations, continued this year. Under the direction of Dr. Allyson Bennett, new methodologies were initiated to test monkey cognitive skills using a computerized joystick system. The methodology allows investigators to vary experimental demands to test specific parameters of cognition in nonhuman primates. Studies compared subjects reared in one of three social conditions: in social groups with adults present (parent-reared) and in one of two social groups without adults present (peer-only-reared, i.e., chronic peer-access) or (surrogate-peer-reared, i.e., daily exposure but limited to only a few hours). Results showed that subjects reared with their parents learned new cognitive tasks rapidly. Peer-only-reared subjects learned the same cognitive tasks, but at a slower rate than the parent-reared subjects. On the other hand, even after a large number of sessions, the surrogate-peer-reared subjects failed to learn the cognitive tasks. In a subsequent study, Dr. Bennett found that monkeys with low CSF 5HIAA concentrations were more likely to engage in potentially risky behaviors when a reinforcer was present. When presented with an opaque black box that was baited with reinforcing food, monkeys with low CSF 5HIAA concentrations were more likely to quickly touch the box and spent more time in close proximity to the box. When the box was constructed out of material that allowed the subjects to see inside, there was no difference between the monkeys with high and low CSF 5HIAA concentrations in the time to touch or sit in close proximity to the box. CSF HVA, on the other hand, was not related to time to approach the boxes, but after the subjects approached the stimulus, it was related to the time spent manipulating both the clear and opaque boxes.

Measurements of HRV are widely used to assess both central and peripheral nervous system functions. An important neuromodulator affecting reflex control of heart rate is serotonin. In a series of studies with Dr. Paolo DePetrillo, we found that monkeys with low CSF 5HIAA concentrations exhibited high HRV, suggesting reduced temporal regulation of the autonomic system in subjects with low CSF 5HIAA concentrations. In a second series of studies conducted with Drs. Salem and Hibbeln (LMBB), we continued our investigation of the role of essential fatty acids (EFA) in CNS serotonin function by assessing heart rate variability in subjects fed diets devoid of or rich in EFA. Subjects fed a diet high in EFA as infants were shown as adolescents to exhibit higher heart rate variability when compared to subjects fed a diet low in EFA, suggesting the importance of EFA in normative development.

In most of our nonhuman primate studies to date, the principle measure of CNS serotonin function has been the removal of CSF to assay for serotonin metabolites. In humans, the invasiveness of this procedure clearly precludes frequent sampling and makes it especially difficult to perform such studies in children. As an alternative, the prolactin response to fenfluramine has been widely used to assess CNS serotonin function. However, obtaining a prolactin response after the fenfluramine challenge is also invasive. Not only must fenfluramine be injected, typically, blood is sampled every 30 minutes for up to 5 hours following fenfluramine administration. Prolactin is also found in the saliva. We hypothesized that salivary prolactin concentrations would correlate positively with CSF 5HIAA and our studies showed they did. These findings support the possibility of using salivary prolactin concentrations as an index of CNS serotonin turnover in humans.

A growing body of research indicates that the 5HT system shows circannual seasonal variation in humans. Seasonal changes of CSF 5HIAA concentrations indicated that levels were significantly increased in the fall (October and November), the height of the breeding season, with no evidence of differences between winter and spring. This finding connotes that like humans, nonhuman primates show seasonal variation in CNS serotonin function, thus suggesting an evolutionary basis for seasonal variation.

RESEARCH HIGHLIGHTS: One of the most powerful advantages of our primate model is the capacity to closely control and investigate the role of genes and environment using a developmental paradigm.

In an analysis of factors affecting alcohol consumption in nonhuman primates, we were able to show that age, sex, drinking conditions, rearing background and strain were important variables influencing alcohol consumption by utilizing for the first time a large population sample. When these important variables where controlled, heritable influence contributions accounted for about 30% of the variance in alcohol consumption in macaques.

In our assessment of the contribution of genetic and environmental variables on development in macaque infants, heritability values significantly greater than zero ($p < .05$) for State Control and Orientation clusters were found on Days 14 and 30, on Day 14 for the Activity cluster, but not for Motor Maturity. Heritability values changed with age, with the pattern of change differing across clusters. We also found that monkeys possessing a length variation in the serotonin transporter gene were deficient in the neonatal measures designed to measure CNS maturity and development.

THROMBOCYTE-MAO (trbcMAO) is, in part, genetically determined and is stable throughout life. There are a number of studies showing a correlation between trbcMAO and personality traits such as sensation seeking and impulsiveness, which in turn underlies connections between low trbcMAO and vulnerability for substance abuse and violent behavior. We found a correlation between trbcMAO and CSF 5HIAA in nonhuman primates. Moreover, we found a positive correlation between trbcMAO, impaired social dominance and excessive alcohol intake. These findings suggest that trbcMAO may act as a biological marker for central serotonin function related to social functioning and excessive alcohol consumption.

We administered a non-peptide CRH type 1 receptor antagonist, antalarmin, to monkeys under stress and showed that antalarmin significantly reduced indices of anxiety while enhancing environmental exploration, a behavior that typically occurs in the context of a non-threatening environment. These data demonstrate a significant role for CRH in regulation of the stress system in primates and suggest that CRH antagonists may be helpful in treating human disorders associated with stress system dysregulation such as excessive alcohol consumption, as well as anxiety disorders and depression.

New methodologies were initiated to test monkeys' cognitive skills using a computerized joystick system that allows variation in experimental demands to test specific parameters of cognition. Results showed that parent-reared subjects learned new cognitive tasks rapidly; peer-only-reared subjects learned the cognitive tasks, but at a slower rate and the surrogate-peer-reared subjects failed to learn the cognitive tasks, even after a large number of sessions. Findings of a subsequent study suggests that monkeys with low CSF 5HIAA concentrations are more likely to engage in potentially risky behaviors when a reinforcer was present.

The principle measure of CNS serotonin function is removal of CSF to assay for serotonin metabolites. We hypothesized that salivary prolactin concentrations would correlate positively with CSF 5HIAA and found this to be true. These findings support the possibility of using salivary prolactin concentrations as an index of CNS serotonin turnover in humans. We found that like humans, nonhuman primates show seasonal variation in CNS serotonin function that suggests an evolutionary basis for seasonal variation in CNS serotonin function.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Through the use of the primate model, we have been able to closely control and investigate the role of genes and environment in alcohol abuse using a developmental paradigm. These findings suggest that both genetic and environmental variables play important roles in type II-like behavior and CNS serotonin function. New studies suggest that CNS serotonin could be evaluated using saliva.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00287-09 LCS

Title: STRESS AXIS ACTIVATION, ETHANOL AND SITE-SPECIFIC CNS NEURODEGENERATION

Staff Years: 2.3

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Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: mental health, stress axis, neuroscience, CNS, neurodegeneration, neuroprotection, glucocorticoids, ethanol, neuroendocrine

Summary: Consumption of moderate to large amounts of ethanol (Et) activates the hypothalamic-pituitary-adrenal axis (HPAA). Activation of the HPAA or hypercortisolism accompanies both short- and long-term consumption of Et and the Et withdrawal syndrome. Alcoholics often present with a pseudo-Cushing's syndrome in which some 17-40 percent of alcoholics do not respond to the dexamethasone suppression test during the first week of abstinence, suggesting an ongoing hypercortisolemic state in this group of patients. Since a relative state of elevated glucocorticoids, e.g., chronic continuous or chronic intermittent, can lead to neural changes and even cell death, particularly in the hippocampus, the progressive loss of cognitive capacity in many alcoholics may be due, in part, to hypercortisolemia and subsequent irreversible neural damage in the hippocampus and other areas of the CNS. Using an intragastric cannulated rodent model and short-term (4 days) intermittent or binge-type Et administration, we have demonstrated site-specific CNS neurodegeneration in the dentate gyrus of the hippocampus, the entorhinal cortex and the piriform cortex. The observed Et-induced neurodegeneration was functionally validated as noted by the decline in learning and memory capacity in the Et-treated animals in the hippocampal-dependent Morris water maze test. Ongoing efforts to define the mechanism of Et's cytotoxicity continue. Surprisingly, the co-administration of glutamate receptor subtype antagonists or calcium uptake blocking drugs with Et are not neuroprotective, which argues against an excitotoxic basis for the neurodegeneration; however, elevated glucocorticoids exacerbate the Et-induced neurodegeneration presumably through excitotoxic mechanisms. To date the most potent cytoprotective agent in the binge-type rodent model has been shown to be furosemide (FUR), an anion transport inhibitor; however, our finding that LY-644,711, bumetanide and SITS, which are drugs with mechanisms of action similar to FUR, are not neuroprotective would argue against a primary ionic, edema-based mechanism of neurotoxicity. Finally, with the knowledge that certain cannabinoids are neuroprotective we co-administered cannabidiol with Et and found an attenuation of neurodegeneration. Since *in vitro* studies have demonstrated that cannabidiol blocked glutamate-NMDA, -AMPA or -kainate receptor-mediated toxicity, it would appear that the cannabidiol site of action is downstream of receptor activation and perhaps has a generalized metabolic or antioxidant mechanism of neuroprotection. The ability of cannabidiol to protect against the toxicity of reactive oxygen species may underlie its cytoprotection in our binge-type Et rodent model.

RESEARCH HIGHLIGHTS: There is widespread agreement that chronic, excessive alcohol consumption leads to learning and memory dysfunction through the cytotoxic action of alcohol on the brain. Using a binge-type alcohol rodent model, we observed site-specific neurodegeneration in the hippocampus and entorhinal cortex. Using a hippocampal-dependent learning paradigm, we found permanently impaired short- and long-term memory in our binge-type treated animals. Elevated

glucocorticoids exacerbate the alcohol-induced damage, whereas, antiedematous and antioxidant compounds offer neuroprotection.

SIGNIFICANCE TO BIOMED RESEARCH & PROGRAM: The site-specific neural damage observed in the binge-type alcohol rodent model and the demonstrated decline in memory and learning establishes a cause to effect relationship between alcohol-induced neurocytotoxicity and reduced cognitive ability. A reliable animal model for one of the most vulnerable alcohol groups, namely the binge drinker, should facilitate an understanding of the mechanism of alcohol-induced brain cytotoxicity and the development of cytoprotective therapeutics.

FY 1999 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1998 - 30 SEPTEMBER 1999)

LABORATORY OF MEMBRANE BIOCHEMISTRY & BIOPHYSICS

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NATIONAL INSTITUTES OF HEALTH

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The first studies of essential fatty acids (EFA) in alcoholics and smokers *in vivo* have been performed within the Office of the Chief. In addition, our studies of infants and adults report some of the first basic studies in the normal case. Highly sensitive and selective methodology has been developed within our research group allowing for the safe and non-invasive assessment of EFA metabolism *in vivo*. This approach takes advantage of the stable isotopically labeled EFA, deuterated linoleic and linolenic acids, along with negative, chemical ionization gas chromatography/mass spectrometry (GC/MS) to simultaneously measure the precursor and product fatty acids.

In human infants in the first week of life, our results indicate a general capacity to perform 18-carbon EFA metabolism to 20 and 22-carbon end products for both the n-3 and n-6 pathways. There was a surprising inverse relationship between gestational age and metabolic capacity that was strongest for docosahexaenoic acid (DHA). This is consistent with the rapid formation of neural membranes for which DHA is an important structural constituent during the brain growth spurt in the third trimester of human development. The rate of conversion in these infants is slow and appears inadequate to support the amount of DHA required for optimal brain and other organ development. We concluded, therefore, that infant formula must have preformed DHA added to support optimal nervous system growth and function; current formulas in North America are devoid of this nutrient, although they contain small amounts of the DHA precursor, alpha-linolenic acid.

Our metabolic studies were also performed in human adults. Our findings indicated that a diet low in n-3 fats leads to an increase in their formation and transport to the plasma compartment. Smokers have increased activity in this regard. Smoking alcoholics have a further marked increase in the deuterium enrichment of plasma DHA. Alcoholics with fibrosis have greater metabolic activity in this regard than do unaffected alcoholics, based on liver histopathology. Both smoking and alcohol are oxidative challenges that are hypothesized to lead to increased EFA degradation. The apparent greater level of formation/transport of the fatty acids may, in part, compensate for these losses. When the intensity of the alcohol challenge is too great, with respect to its frequency and dosage, metabolism cannot keep pace with the increased catabolism and tissue levels fall, consistent with our findings in many species.

Adult studies of EFA metabolism have been extended to the case of neurological disease states, for the first time. In collaboration with Dr. William Connor and colleagues, patients with Retinitis Pigmentosa have been given deuterated 18-carbon EFAs and their plasma metabolites quantified over an extended period. The amount and enrichment of isotope-labeled n-3 fatty acid metabolites, particularly DHA, was increased in Usher's II type patients but decreased in non-Usher's disease relative to normal controls. The increased metabolism observed in Usher's disease may be surprising in view of the decreased levels of DHA observed in the circulation and, presumably, in the retina. However, as was the case in alcoholics, increased fatty acid metabolism may be coupled to increased catabolism related to the disease process. However, the opposite response in the non-Usher's patients indicated a quite distinct etiology of this illness.

In concert with these studies of metabolic capacities of infants for AA and DHA formation *in vivo*, a series of studies was performed to examine the consequences of DHA depletion in the rat nervous system. Diets with varying amounts of n-3 and n-6 precursors and DHA were formulated and fed to rats over three generations so that by F2, the brains and retinas had graded levels of DHA. Brain function was assessed by behavioral means using olfactory-based discrimination and spatial tasks. The n-3 adequate groups performed better in retaining a spatial memory in water maze tasks than did the n-3 inadequate animals. Animals with low brain or olfactory bulb levels of DHA made significantly more mistakes in acquiring an olfactory discrimination task. When the animals were over-trained, n-3 adequate animals appeared to learn the set as they acquired a strategy that enabled them to make zero or one error after an information trial. These studies provided clear evidence that lack of an adequate n-3 fatty acid dietary source in early

development, and persisting through adulthood, results in a low brain DHA level and this has impact on memory and learning related brain functions.

In order to assess the behavioral underpinnings of the differences in olfactory learning and spatial memory, control experiments were performed. There was little or no difference in measures of general activity or in the plus maze either in the normal state or after restraint stress. There was no difference in the ability of rats to respond to a diluted odor, even when masked with a higher level of a second odor, indicating that there was no loss in olfactory sensory ability. Also, a progressive-ratio-licking task, in which the water-deprived animals worked for a water reward until extinction, showed no difference between dietary groups.

In a closely related line of inquiry, an attempt was made to pioneer the quantification of alterations in brain morphology in n-3 deficient animals with low brain DHA. Preliminary results were encouraging, suggesting a loss of neuronal area in the hippocampus of n-3 deficient rats. There were no differences in cell number or density or the areas of the hippocampus between dietary groups.

In a further development in the area of nutritional neuroscience, a method invented by Hoshiba was adapted for the use in the study of EFA during rat development. This system, termed "automatic feeding", makes possible the complete control of any nutrient during the entire life cycle of a rat when suitable diets are constructed. Progress has been made in the construction of artificial rat milks appropriate for use with this apparatus and for the control of EFA levels.

UNIT OF MOLECULAR BIOLOGY

Our efforts focus on two main projects: the molecular regulation and functional importance of ethanol-inducible cytochrome P450 2E1 (CYP2E1) and the preparation and characterization of transgenic mice carrying the human aldehyde dehydrogenase 2 (ALDH2) mutant and knock-out mice deficient in mouse ALDH2 gene. Using YH439, a synthetic inhibitor of CYP2E1 gene transcription, we observed that a 37 kDa protein-acetaldehyde adduct, absent in the control animals, was present in alcohol-treated rat livers. Our immuno-histochemical analysis revealed that this adduct protein is localized in the pericentral region, where CYP2E1 is mainly expressed in the liver. The level of this adduct protein highly correlated with that of CYP2E1 ($r = 0.95$). In contrast, there were no significant changes in the catalytic activities and contents of alcohol dehydrogenase or aldehyde dehydrogenase isozymes. These results strongly indicate that the 37 kDa protein-acetaldehyde adduct could be produced by the CYP2E1-dependent ethanol metabolism.

We also continued to study the molecular mechanism of apoptosis or necrosis caused by a variety of CYP2E1 substrates, using *in vivo* animal studies as well as *in vitro* studies of cultured cells. C6 glioma cells or neuro2A cells were used as a model system, since these cells are known to contain CYP2E1, despite extremely low levels of CYP2E1 expression in these cells, compared to that in the liver or kidney. By using these cells, we markedly reduced labor, lab resources and time as transfection with the CYP2E1 cDNA was not needed nor was the extended selection process. Unlike the artificial transfection of the exogenous cDNAs, these cells provide us with continuous production of CYP2E1, which is critically important for the metabolic activation of its substrates.

Our results suggest that apoptosis caused by various CYP2E1 substrates such as ethanol, CCl_4, 4-hydroxynonenal, or acetaminophen (Tylenol®) is mediated through a selective and transient activation of c-*jun N*-terminal protein kinase (JNK) without activation of other mitogen activated protein kinases, involved in immediate early signal transduction. The selective activation of the JNK appears to be important for cell death, since the transfection of a dominant negative mutant, SEK1 KR, an immediate upstream kinase of the JNK, not only blocked the JNK activation but also reduced the rate of apoptosis caused by CYP2E1 substrates. The beneficial effects of various CYP2E1 inhibitors on cell death are being evaluated.

The mitochondrial ALDH2 is the major ALDH isozyme involved in acetaldehyde metabolism. A single nucleotide substitution (G to A), with a subsequent change in one amino acid (Glu487Lys), leads to dominant inactivation of ALDH2 activity. This genetic polymorphism results in the "flushing" response observed in many Asian people after alcohol intake. Although the ALDH2-2 allele has been shown to have a protective role against alcoholism, the significance of this enzyme is still unclear. To further

investigate the physiological role of ALDH2 in alcohol-mediated tissue damage, drinking behavior and metabolism of endogenous and exogenous substrates, we produced transgenic mice carrying the human ALDH2 variant (Haldh2-2). Currently, we established two independent lines of Haldh2 transgenic mice. Our data, so far, indicate that both male and female transgenic mice showed 40-50% higher acetaldehyde levels in the livers than the FVB/N background mice (n=6 for each group, $p<0.01$) at 2 hr after ethanol injection. In order to obtain clear results, we have tried to produce mice deficient in mouse ALDH2 gene by the gene disruption technique. Due to our repeated failures in the past, we have reconstructed a new DNA vector for gene disruption. This new DNA construct is being used for electrophoration and subsequent screening for the positive embryonic stem cells with our knockout cassette.

UNIT OF METABOLISM

Having automated part of our analyses over the past year, we continued our investigations of the effects of ethanol on the control of metabolic systems. It is known that l-glutamine is the preferred substrate for gut energy metabolism and it is widely believed that its metabolism is required for the maintenance of gut integrity. This year, we completed a study showing that the metabolic product of hepatic ethanol metabolism, namely acetate, causes a complete cessation of l-glutamine metabolism by the gut. The consequences of such a cessation of what is thought to be an essential nutrient for gut remains to be determined. In a parallel study, we examined in the liver, *in vivo*, the effects of ethanol induced hyperpolarization from -28 to 50 mV on the flux and the energy gradients of amino acid and their ionic cofactors in liver. We determined the apparent elasticity of all of the major amino acid transport systems in relationship to the changes in the overall system. We found that l-alanine transport by system A was unaltered by hyperpolarization whereas the transport of l-glutamine by system N was altered during ethanol metabolism in both direction of flux and the energy of its gradients.

In a collaborative study (conducted with the Department of Neurology, Tottori University and the Department of Biochemistry, Oxford University) we determined the effects of alteration in substrate on model toxicities of Alzheimer's and Parkinson's disease in neurons in culture and showed that toxicity could be altered *in vitro* by change in substrate.

SECTION OF FLUORESCENCE STUDIES

Our studies are targeted at determining the role of membrane acyl chain composition on the function of G protein-coupled signaling systems. Receptors for many neurotransmitters, sight, taste and smell are among the members of this superfamily of receptors. Studies in our Section have demonstrated that the formation of the active form of rhodopsin was favored in more highly unsaturated phospholipid membranes. The highest levels of rhodopsin activation were seen in membranes containing DHA acyl chains. In addition, these bilayers showed the greatest resistance to the inhibitory effects of added cholesterol and were most sensitive to the enhancing effects of ethanol.

The effect of osmotic stress and ethanol on the formation of the active form of light activated rhodopsin, meta-rhodopsin II (MII), was determined in order to asses the effect of body water activity, as determined by the osmotic levels in the plasma, on the action of ethanol. Initial experiments demonstrated that MII formation was sensitive to osmotic stress and indicated that about 35 water molecules are released when MII is formed from its inactive precursor MI. Addition of ethanol showed a synergistic effect, in which the potency of ethanol was enhanced by about 2.5 fold relative to measurements made in the absence of osmolytes. In the presence of ethanol, the number of water molecules released upon MII formation increased from 35 to about 49, suggesting that there is a pool of about 15 water molecules associated with MII that are sensitive to the presence of ethanol.

The effect of acyl chain composition and ethanol on the integrated visual transduction pathway was also studied. This is accomplished by measuring the activity of the effector enzyme in this pathway, the cGMP specific phosphodiesterase (PDE), in native disks and in reconstituted membranes. These studies show that the level of PDE activity is greater in a mixed chain phosphatidylcholine with a DHA acyl chain than in one with a monounsaturated (oleate) acyl chain. In contrast to our results with rhodopsin, ethanol appears to inhibit the PDE solely through exerting an osmotic effect, rather than through any direct interaction of ethanol with the PDE molecule.

Studies were also undertaken to identify special structural properties that may be imparted to natural membrane by the presence of DHA. This was accomplished by using acyl chain specific, fluorescent probes. Rhodopsin was shown to induce a lateral phase separation and to select a lipid environment of predominantly DHA containing lipids.

Our studies show that receptor activation is sensitive to both body water activity and that osmolyte concentrations of the order of those found in plasma increase the potency of ethanol by about 2.5 fold. In addition, there is evidence for a receptor-induced formation of a lateral membrane domain enriched in DHA-containing lipids.

SECTION OF NUCLEAR MAGNETIC RESONANCE

We investigate the influence of the lipid matrix on the function of neural receptor proteins, in particular, the influence of high concentrations of polyunsaturated fatty acids like the six-fold unsaturated docosahexaenoic acid (DHA). Several lines of evidence suggest that high DHA concentrations are necessary to achieve full activity of certain neural membrane receptors. We applied recent developments in NMR spectroscopy to the study of membrane structure and dynamics to obtain a better description of membrane properties that modulate membrane receptor function. Modern NMR techniques require only milligram size samples of membrane material and are compatible with investigation of complex biological membranes containing mixtures of lipids and proteins at physiological conditions. In many instances, we are able to pinpoint location of membrane molecules in the lipid matrix with atomic resolution. For example, we determined that short chain alcohols like ethanol locate preferentially near the membrane-water interface and lower interfacial energy of lipids and proteins. These techniques enable a more detailed description of membrane biophysical properties, including parameters that describe the energy of elastic membrane deformation that can be linked to the membrane receptors' structural transitions during excitation.

The alteration of membrane mechanical properties is one possible role of lipid polyunsaturation. There has been controversy concerning the nature of the perturbation which DHA chains induce on membrane elasticity. The six methylene-interrupted cis double bonds within DHA's 22 carbon unit reduce the number of degrees of freedom for structural transitions. This led to the suggestion that these chains have a specific rigid angle-iron or helical-like conformation. This is at variance with our studies of membrane lateral compressibility, which indicate exceptionally high deformability of DHA chains when under lateral tension. Using a magic angle spinning NMR experiment which re-couples ^{13}C-^{1}H dipolar interactions, we obtained assigned DHA order parameters, and we measured average DHA chain length and molecular area of phospholipids with DHA chains by x-ray diffraction. With this information, we now have some constraints by which to examine conformations of DHA, proposed by molecular modeling studies, to determine their correlation with experimental data. The results suggest that DHA chains in membranes prefer looped conformations and undergo rapid structural transitions, providing increased flexibility to receptor-rich neural membranes. Moreover, the efforts of our team are beginning to provide an explanation in which the biophysical properties and functions of membrane lipids can be understood in terms of their degree of unsaturation, discrimination that nature clearly makes.

SECTION OF MASS SPECTROMETRY

It has been proposed that an important mechanism underlying many of the effects of ethanol is its capacity to alter the metabolism of polyunasaturates. The principal objective of our research is to elucidate the biological and metabolic functions of the principal polyunsaturated fatty acids, docosahexaenoic acid (DHA) and arachidonic acid (AA) in the nervous system with particular reference to their modulation by ethanol. We previously found that docosahexaenoic acid affects the survival of neuronal cells and the accumulation of phosphatidylserine (PS). During this period, we continued to investigate mechanisms underlying the observed protective effect by examining the signaling pathways leading to cell death and survival.

Docosahexaenoic acid prevented the apoptotic cell death of Neuro 2A and PC-12 cells only after the incorporation of DHA into phospholipids. After a prolonged incubation, this fatty acid was accumulated primarily in PS, suggesting that DHA as a membrane PS may play an important role for the protective

effect. *In vitro* biomolecular interaction assays indicated that translocation of Raf-1 kinase, which is essential for survival and normal cell growth, is dependent on the PS concentration. We found that in neuronal cells where DHA is known to be concentrated, the PS content is especially high and the accumulation of PS was significantly affected by the DHA status. A dramatic reduction of PS content was observed in neuronal membranes when n-3 fatty acid deficiency existed during the prenatal or developmental period. This phenomenon appeared to be limited to neuronal cells, since other tissues where DHA content is low contained less PS and PS accumulation was not affected by n-3 deficiency. These results suggested that the biological significance of DHA may reside, at least partly, with the role as a modulator of PS accumulation, and neuronal cells may require higher PS contents for survival. The loss of polyunsaturated PS caused by depleted DHA supply may have significant implications for neuronal dysfunction.

We also observed during this reporting period that polyunsaturated fatty acid metabolism through lipoxygenation was significantly reduced by melatonin. This reduction was accompanied by the decreased expression of 12-lipoxygenase at both protein and mRNA levels, suggesting that melatonin may be an endogenous negative modulator of polyunsaturate metabolism. As a continuing effort to determine trace levels of neurosteroids from biological fluids using a GC/MS/NCI technique, we were able to detect species differences in $GABA_A$ active neurosteroids present in cerebrospinal fluid or plasma.

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Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00003-07 LMBB

Title: NMR INVESTIGATIONS OF CELL MEMBRANE STRUCTURE

Staff Years: 5.2

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Sample Type: Neither human subjects nor human tissues

Keywords: cognitive deficits/central nervous system, NMR spectroscopy, cell membrane, polyunsaturation, docosahexaenoic acid, alcohol

Summary: The objectives of this project are: (1) to investigate the interaction of alcohol with proteins and lipids in biological membranes; (2) to study structure and dynamics of membranes composed of lipids with polyunsaturated fatty acids such as docosahexaenoic acid (DHA) 22:6n-3; and (3) to study lipid-protein interactions related to alcoholism and lipid polyunsaturation.

We obtained direct evidence by NMR that ethanol interacts preferentially with the lipid-water interface of membranes. Ethanol's interactions are driven by the opportunity for hydrogen bonding and hydrophobic interactions. We quantitated ethanol binding to membranes composed of lipids and proteins at the physiological ethanol concentration of 20 mM by headspace gas chromatography. This method is ideally suited for partitioning studies because it is non-perturbing. Under physiological conditions, nearly 10% of total ethanol is bound to the interfaces of lipids and proteins. NMR measurements indicate that free and bound ethanol molecules are in rapid exchange, and that ethanol passes through membranes at rates that are only slightly lower than permeation rates of water. Interfacial binding of ethanol raises effective ethanol concentrations at surfaces but lowers its concentration in the electrolyte solutions of living organisms. The interface location of ethanol lowers interfacial energy of lipids and proteins. In lipid membranes, this results in an increase of area per lipid molecule and a disordering of lipid hydrocarbon chains. Ethanol-induced chain disordering is smaller in polyunsaturated bilayers, most likely because polyunsaturated hydrocarbon chains already occupy a larger area per molecule and are therefore less sensitive to ethanol-induced disordering. The ethanol molecules at the lipid-water interface block pathways for water diffusion through lipid bilayers as seen in decreased rates of water permeation.

The membranes of brain synaptosomes and retinal rod outer segments contain 30-50 mol percent of the six-fold unsaturated docosahexaenoic acid (DHA) as lipid hydrocarbon chains. One possible role of DHA is to alter membrane mechanical properties important for activity of receptor proteins. There is controversy as to the nature of the perturbation which DHA chains induce on membrane hydrocarbon order. The six methylene-interrupted cis double bonds within DHAs 22 carbon unit reduce the number of degrees of freedom for structural transitions, which led to the suggestion that these chains have a specific rigid conformation such as angle-iron or helical. However, direct measurements of DHA chain order

parameters reveal a different picture. Using a magic angle spinning NMR experiment which re-couples 13C-1H dipolar interactions, assigned DHA order parameters were obtained and dimensions of the DHA chain unit cell were determined by x-ray diffraction. The results suggest that DHA chains in membranes prefer looped conformations and undergo rapid structural transitions, providing increased flexibility to receptor-rich neural membranes. We developed quantitative methods for interpretation of NMR NOESY cross-relaxation rates between lipid resonance. In addition to providing information on lipid structure, these rates are sensitive to the dynamics of membrane reorganization in the correlation time range from pico- to microseconds. The comparison of experimental rates and rates from molecular dynamic calculations suggests that distance variation between protons, caused by lateral diffusion of lipid molecules, is the primary mechanism of cross-relaxation in lipids. The analysis quantifies the high degree of molecular disorder in biological membranes, showing a finite probability of close approach between even the most distant segments of neighboring lipid molecules (e.g., the methyl groups in the choline headgroup and the terminal methyl groups of the fatty acid chains). Intermolecular cross-relaxation rates are an ideal tool to study lateral lipid organization in the liquid-crystalline phase of lipids. In homogeneous lipid distribution, preferences in the interaction of lipid species, as well as preferences in the location of substances that incorporate into membranes, can be detected.

The behavior of the cytolytic peptide fragment 828-848 (P828) from the carboxy-terminus of the envelope glycoprotein gp41 of HIV-1 in membranes was investigated by solid state 2H NMR on P828 with the selectively deuterated isoleucines I3, I13, I16, and I20. The data are consistent with partial penetration of the N-terminal peptide region into the hydrophobic core of the membrane, while the C-terminal portion of the peptide remains near the lipid/water interface. Peptide incorporation results in a significant reduction of lipid chain order toward the bilayer center, but only a modest reduction near the lipid glycerol. In addition, the structure of the peptide was investigated, free in water and bound to SDS micelles, by high resolution NMR. P828 is unstructured in water but exists in a flexible, partially helical conformation when bound to negatively charged liposomes or micelles. The flexible helix covers the first 14 residues of the peptide, whereas the C-terminus of the peptide appears to be unstructured. The peptide-induced changes in lipid chain order profiles indicate that membrane curvature stress is the driving force for the cytolytic behavior of P828.

RESEARCH HIGHLIGHTS: The alteration of elasticity of neural membranes by the number of double bonds per fatty acid is one possible role of lipid polyunsaturation. Membranes contain a matrix of lipid molecules with hydrophilic ("water-loving") headgroups and hydrophobic ("water rejecting") fatty acid chains. Membranes respond to external perturbations like elastic rubber bands. With nuclear magnetic resonance spectroscopy and x-ray diffraction, we are able to follow the elastic deformation of membranes under tension, including the changes in structure and motions of lipid hydrocarbon chains. The results suggest that polyunsaturated chains in membranes prefer flexible, looped and helical structures that provide increased flexibility to receptor-rich neural membranes.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: A common perception of polyunsaturated chains is that they are inflexible or stiff due to the presence of motionally restricted double bonds. In contrast, our study of membrane lateral compressibility indicates exceptionally high deformability of DHA chains when under lateral tension. Perhaps, this is the missing link that explains why neural receptors are sensitive to lipid composition.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00036-13 LMBB

Title: REGULATION AND BIOLOGICAL ROLE OF ETHANOL INDUCIBLE CYTOCHROME P450 2E1 (CYP2E1)

Staff Years: 2.25

Principal Investigator: Song BJ, PhD (MB, LMBB, NIAAA)

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NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: neurosciences, health & behavior, molecular genetics, cirrhosis, hepatology, cell and molecular biology, alcohol, metabolism, apoptosis

Summary: We previously demonstrated multiple regulatory mechanisms for ethanol inducible cytochrome P450 2E1 (CYP2E1): induction via transcription, mRNA stabilization, activation of mRNA translation and protein stabilization, suppression via transcription, mRNA degradation and protein degradation. We recently reported a transcriptional suppression of the CYP2E1 gene by an exogenous compound, YH439. During FY99, we studied the biological role of CYP2E1 in acetaldehyde-protein adduct formation in rats chronically treated with alcohol. The 37 kDa acetaldehyde adduct protein was detected in alcohol-treated rats while it is absent in pair-fed control animals. During ethanol treatment, the level of CYP2E1 activity was significantly elevated while other enzymes, such as alcohol or aldehyde dehydrogenase, involved in alcohol or acetaldehyde metabolism were unchanged. Co-treatment of YH439 with ethanol markedly reduced the level of the 37 kDa adduct, suggesting that this adduct is most likely produced by a CYP2E1-dependent mechanism. In addition, we tested the protective effect of YH439 on the apoptosis of C6 glioma cells caused by acetaminophen (AAP, Tylenol) and other CYP2E1 substrates. The major advantage of C6 glioma cells over other established cell lines is that we don't need to transfect CYP2E1 cDNA and select the stable transformants prior to use, since CYP2E1 is known to be expressed in C6 glioma cells despite its very low level of expression. Treatment of AAP or other CYP2E1 substrates, including ethanol, caused time and dose-dependent apoptosis of C6 cells as evidenced by classical DNA fragmentation. In C6 cells, c-jun N-terminal protein kinase activity (JNK) was selectively and transiently activated after AAP treatment. The activity of p-38 protein kinase or mitogen activated protein kinase remained unchanged. The selective activation of the JNK pathway by AAP is similar to the mechanisms of cell death caused by 4-hydroxynonenal (HNE) or carbon tetrachloride, and other substrates of CYP2E1. Furthermore, pretreatment of YH439 (10 uM) of C6 cells not only reduced the CYP2E1 level but also prevented the apoptosis observed at 18- and 36-hr post-AAP treatment. Consistent with the *in vitro* data, JNK was selectively activated in mouse liver treated with AAP or carbon tetrachloride. To further elucidate the relationship between the c-jun kinase activation and apoptosis of cultured cells, we are studying the role of various caspases upon treatment of CYP2E1 substrates, including ethanol and arachidonic acid. In addition, changes in the level of DNA-adducts in the CYP2E1-transfected cells and C6 glioma cells are being determined by HPLC.

RESEARCH HIGHLIGHTS: We continued to study the biological function of ethanol-inducible cytochrome P450 2E1 (CYP2E1) in acetaldehyde-protein adduct formation. A 37 kDa protein-acetaldehyde adduct, absent in control rats, was detected in alcohol treated animals and its level highly correlated with those of CYP2E1 activity and content but not with alcohol or aldehyde dehydrogenase involved in alcohol or acetaldehyde metabolism. Co-treatment of ethanol with YH439, an inhibitor of CYP2E1, markedly reduced the level of the 37 kDa adduct, suggesting that this adduct is most likely produced by a CYP2E1-dependent mechanism. In addition, we studied the protective effect of YH439 on the programmed death of cells by acetaminophen (AAP, Tylenol) and other CYP2E1 substrates.

Pretreatment of YH439 of the cultured cells not only reduced the CYP2E1 level but also prevented the cell death observed at 18- and 36-hr post-AAP treatment. These results indicate the important role of CYP2E1 and the potential benefits of CYP2E1 inhibitors in treating or preventing alcohol and chemical-mediated cell or tissue damage.

SIGNIFICANCE TO BIOMED RESEARCH AND THE PROGRAM: We studied the molecular mechanism to ascertain how alcohol and other potentially harmful substances can cause tissue or cell damage. We hope that our molecular studies will lead to development of potential therapeutic agents against various disease states including alcohol-mediated tissue damage. Our findings may also help to increase public awareness about viable strategies for preventing or reducing the incidence of alcohol-related diseases.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00069-08 LMBB

Title: SODIUM POTASSIUM ATPase--FUNCTION AND REGULATION, ALCOHOLISM, NEUROSCIENCE

Principal Investigator: Salem Jr N, PhD (LMBB, NIAAA)

Summary: TERMINATED

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00072-08 LMBB

Title: FLUORESCENCE STUDIES OF BIOPHYSICAL PROPERTIES OF POLYUNSATURATED PHOSPHOLIPIDS

Staff Years: 2.2

Principal Investigator: Litman BJ, PhD (FS, LMBB, NIAAA)

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Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: neurosciences, nutritional disorders

Summary: Neuronal and retinal tissue are high in phospholipids containing one or two long chain polyunsaturated acyl chains. A further refinement of our analysis of the time-resolved anisotropy decay of the hydrophobic fluorescence probe diphenylhexatriene (DPH) allows a measure of the acyl chain packing order in the membrane interior vs the portion of the chain closer to the interfacial region. Polyunsaturated acyl chains result in the highest degree of acyl chain disorder and produce the highest values of acyl chain packing free volume, a parameter which promotes the greatest degree of formation of the activate conformation of the G protein-coupled receptor, rhodopsin. Cholesterol has been shown to decrease both acyl chain packing free volume and rhodopsin activation. Our studies show that polyunsaturated phospholipids are able to resist the ordering effects of cholesterol and maintain their unique acyl chain packing properties. Recent findings demonstrate that acyl chain packing is increased by increased osmotic stress, due to dehydration of the phospholipid head group. Ethanol on the other hand has an antagonistic effect of decreasing acyl chain order. These effects are important in understanding *in vivo* mechanisms of alcohol action, since ethanol effects occur in the presence of an osmotic background of plasma. These findings have important implications for integral membrane protein function and suggest that highly unsaturated phospholipids found in retina and neuronal tissue can optimize receptor function, are able to resist the effects of certain compositional variation, such as increases in cholesterol content, and may modulate membrane sensitivity to ethanol.

RESEARCH HIGHLIGHTS: Research on model membrane systems has demonstrated that phospholipids containing polyunsaturated fatty acids are better able to resist cholesterol induced changes in bilayer properties, which are associated with the inhibition of receptor function, to a much greater degree than more saturated lipid bilayers. Our studies indicate that docosahexaenoic (DHA) acid acyl chains best resist the acyl chain ordering effects of cholesterol. Other studies show that these polyunsaturated bilayers are more sensitive to the effect of ethanol than are more saturated bilayers.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Since the retina and nervous system are rich in polyunsaturated lipids, these tissues should be less effected by cholesterol and a potential target for the enhanced effects of ethanol. An inadequacy of dietary omega-3 fatty acids, which results in reduced membrane DHA content, is correlated with both cognitive and visual deficiencies. In addition, DHA containing membranes were found to be most resistant to the effects of cholesterol. These findings highlight the nutritional and health requirements for omega-3 (n-3) fatty acids for optimal function in the nervous system.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00080-06 LMBB

Title: INFLUENCE OF PROTEIN/LIPID INTERACTIONS ON SIGNAL TRANSDUCTION

Staff Years: 3.1

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NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: neurosciences, nutritional disorders, signal transduction

Summary: G protein-coupled receptors are ubiquitous components of signal transduction pathways, including many neurotransmitter systems. This project is designed to assess the role of polyunsaturated phospholipids in modulating G protein-coupled signal transduction and to elucidate the mechanism of action of ethanol in these systems. The visual transduction pathway of the retinal rod photoreceptor is being used as a model system. System properties studied include: (1) the kinetics and extent of formation of metarhodopsin II (MII), the G protein activating form of rhodopsin; (2) MII/G protein complex formation; (3) the rate of G protein activation; (4) cGMP phosphodiesterase (PDE) activation; and (5) the GTPase activity of the G protein. Both functional measures in the transduction pathway and acyl chain packing properties of the phospholipid bilayer are being investigated. Current studies demonstrate the importance of membrane hydration in modulating both rhodopsin activation and integrated activity in the signaling pathway. MII formation involves the release of some 34 water molecules, a process, which is enhanced by increased osmolality. An important result of these studies is that in the physiological concentration range of ethanol and at the osmolality of human plasma, the effect of ethanol in enhancing MII formation is increased by a factor of about 2.8. *In vitro* effects observed in the 100 mM to 280 mM ethanol concentration range are usually considered to be outside the physiological range of interest. However, when measurements are made in the presence of an inert osmotic background, the equivalent results may well be observed in the physiological range of ethanol concentration. In contrast, ethanol has little effect of the PDE activity at physiological levels, but exhibits a moderate osmotic effect at high concentrations, which results in PDE inhibition due to reduced water activity. These findings demonstrate the importance of simulating *in vivo* conditions to the greatest degree possible, when attempting to evaluate ethanol potency in *in vitro* experiments. In addition, a generalization of these results suggests that ligand binding and drug partitioning experiments may also be altered dramatically when conducted in the presence of a neutral osmolyte.

RESEARCH HIGHLIGHTS: Our recent studies highlight the importance of membrane surface and protein surface hydration in modulating the activity of proteins in the visual signaling cascade and the potency of ethanol. Increased osmotic strength, which reduces the effective concentration of water, i.e. the water activity, was found to enhance the activation of rhodopsin, while inhibiting the activity of the cGMP phosphodiesterase effector enzyme. A 2.7 fold increase in the potency of ethanol, with respect to enhancing rhodopsin activity, was observed in the presence of 360 mOs of neutral solute. Ethanol demonstrated a mixed mode of action, relative to its effect on rhodopsin, composed of a direct association with both the membrane lipid and rhodopsin. In experiments using mixed docosahexaenoic acid (DHA) and saturated phospholipid model systems, with and without rhodopsin, it was observed that rhodopsin induced a lateral domain formation, wherein it was preferentially surrounded by the DHA containing phospholipid.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: There is a controversy in the literature as to both the mechanism of action of ethanol and the appropriate levels of ethanol to be used in *in vitro* experiments. Our studies demonstrate that, in any signaling system, ethanol may have a complex mode of action rather than a uniform mechanism throughout the signaling system. In addition, the effect on different components of the same system may be in opposite directions. Our studies also indicate that *in vitro* experiments may underestimate the true potency of ethanol, in that they do not take into count the osmolality of plasma, which effects water activity. At osmolalities near that of human plasma, the potency of ethanol was observed to increase by 2.7 fold, demonstrating the importance of simulating the *in vivo* conditions with respect to water activity, when carrying out *in vitro* experiments. The retina and nervous system are rich in polyunsaturated lipids. Our studies show that the G protein-coupled receptor, rhodopsin, induces a lateral phase separation in which it is preferentially surrounded by DHA containing phospholipids. DHA phospholipids were also found to optimize rhodopsin activation. An inadequacy of dietary omega-3 fatty acids, which results in reduced membrane DHA content, is correlated with both cognitive and visual deficiencies. These findings highlight the nutritional and health requirements for omega-3 (n-3) fatty acids for optimal function in the nervous system.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00089-05 LMBB

Title: MEASUREMENTS AND METABOLISM OF NEUROSTEROIDS IN THE CENTRAL NERVOUS SYSTEM

Staff Years: 1

Principal Investigator: Kim H, PhD (MS, LMBB, NIAAA)

Other Personnel: Kevala K, MS (MS, LMBB, NIAAA)
Zhang H, PhD (MS, LMBB, NIAAA)
McLaurin CG (MS, LMBB, NIAAA)
Ratliff CM (MS, LMBB, NIAAA)

NIH Collaborators: None

Other Collaborators: None

Sample Type: Human tissues, fluids, cells, etc.

Keywords: mental health research, neurosciences, anxiety disorders, alcohol withdrawal, mass spectrometry, cerebrospinal fluid

Summary: The principal objective of this study is to determine the effect of ethanol on the metabolism of neurosteroids in the central nervous system (CNS). Neurosteroids such as 5a-pregnane-3a-ol-20-one (5a,3a-THP or Allopregnanolone) and 5a-pregnane-3a,21-diol-20-one(THDOC) have been shown to modulate the GABA/benzodiazepine binding sites and to exert anxiolytic and hypnotic effects. Modulation of these neurosteroids in the CNS by ethanol may be one of the underlying mechanisms for stress-related situations often observed in human alcoholics during withdrawal. The GC/MS-NCI technique that we have established for the measurements of trace levels of neurosteroids in human cerebrospinal fluids (CSF) has been extended to the analysis of human and rat plasma samples in this period. We were able to detect species differences from the levels of pregnenolone, 5a,3a-, 5b,3a-, 5a,3b-THP, androsterone and dihydrotestosterone in human and rat plasma samples. Among the four isoforms of THP, human plasma contains 5a,3a-, 5b,3a- and 5a,3b-THP at 70-150 picogram/ml concentrations while rat plasma contains only 5a,3a- and 5b,3b isomers at the similar concentration range.

RESEARCH HIGHLIGHTS: Human cerebrospinal fluid contains anxiolytic neurosteroid, all-pregnanolone, which can be measured by a sensitive mass spectrometric technique.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM: Measurement of trace levels of neurosteroids during alcohol consumption and withdrawal will allow further understanding of biochemical mechanisms underlying the effect of ethanol on anxiety and depression.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00090-05 LMBB

Title: ROLE OF ALDH2--TRANSGENIC MICE CARRYING ASIAN ALDH2-2 VARIANT ALLELE

Staff Years: 0.5

Principal Investigator: Song BJ, PhD (MB, LMBB, NIAAA)

Other Personnel: Jeong K, DVM, PhD (MB, LMBB, NIAAA)

NIH Collaborators: Gonzalez FJ, PhD (NCI)
Wang T, PhD (LNMR, NCI)

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: health & behavior, cirrhosis, drinking patterns & causes, ethnicity, molecular genetics, transgenic mice, knock-out mice

Summary: Mitochondrial aldehyde dehydrogenase (ALDH2) is the major ALDH isozyme involved in acetaldehyde metabolism. It is well established that a single nucleotide substitution (G to A) which results in the amino acid change (Glu487Lys) leads to dominant inactivation of ALDH2 activity. Our data indicated that the recombinant human ALDH2 variant protein interacted with the mouse ALDH2 protein and dominantly inhibited the activity of the mouse enzyme. The genetic polymorphism is the cause of the flushing response observed in many Asian people following alcohol intake. Although the ALDH2-2 allele has been shown to have a protective role against alcoholism, the physiological role of this enzyme is still unclear. To further examine the physiological role of ALDH2 in alcohol-mediated tissue damage, drinking behavior and metabolism of endogenous and exogenous substrates, we produced transgenic mice carrying the human ALDH2 variant (Haldh2-2). Currently, we have established two independent lines of Haldh2 transgenic mice. Human ALDH2 protein was expressed in all tissues examined and expression of human ALDH2-2 inhibited mouse ALDH2 enzyme activity in transgenic mice. We also observed that the FVB/N background strain mice showed fear, avoidance and escape behavior three days after treatment with 20% ethanol but these changes in behavior were not evident in the transgenic mice exposed to ethanol ($p<0.001$). To correlate the apparent behavioral change with levels of neurotransmitters and to study the role of ALDH2 in endobiotic metabolism, the levels of various monoamine neurotransmitters are being determined by HPLC. Our data also indicate that both male and female transgenic mice showed 40-50% higher acetaldehyde levels in their livers than the FVB/N background mice ($n=6$ for each group, $p<0.01$). We are determining the drinking preference in a two-bottle choice paradigm. Data collected to date indicate that transgenic mice carrying the Haldh2-2 can be a valuable model for studying the role of ALDH in behavior, neurotransmitter metabolism and drinking preference. In order to have clear results for the physiological roles of ALDH2, we have tried to prepare knockout mice deficient in mouse ALDH2 gene using a DNA vector we constructed. After repeated failures to obtain positive ES cells with the knockout vector, we have constructed another DNA vector for gene disruption. The plan is to inject the new DNA vector into ES cells in the near future. The resulting ES cells will be screened for positive ES cells using Southern blot analyses.

RESEARCH HIGHLIGHTS: Currently, we have established two independent lines of transgenic mice carrying the human aldehyde dehydrogenase (ALDH2-2) variant. Human ALDH2 protein was expressed in all tissues examined and expression of human ALDH2-2 inhibited mouse ALDH2 enzyme activity in transgenic mice. Our data also indicate that both male and female transgenic mice showed 40-50% higher acetaldehyde levels in the livers than the control mice ($n=6$ for each group, $p<0.01$). We are determining the pathological effect of acetaldehyde accumulation after alcohol treatment. We are also trying to prepare mice deficient in mouse ALDH2 gene to define the physiological roles of ALDH2 in alcohol mediated tissue damage, neurotransmitter metabolism and drinking preference. A new DNA vector has been constructed and introduced into ES cells. The resulting transformant ES cells are being screened for positive ES cells with the knockout vector.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM: Mitochondrial aldehyde dehydrogenase (ALDH2) is the major ALDH isozyme involved in acetaldehyde metabolism. It is well established that a single nucleotide substitution (G to A) which results in the amino acid change (Glu487Lys) leads to dominant inhibition of ALDH2 activity. This mutation is the main cause of the "flushing" response observed in many Asian people following alcohol intake. Although the ALDH2-2 allele has been shown to have a protective role against alcoholism, the physiological role of this enzyme is still unclear. To further examine the physiological role of ALDH2 in alcohol-mediated tissue damage, drinking behavior and metabolism of endogenous and exogenous substrates, we produced transgenic mice carrying the human ALDH2 variant (Haldh2-2) and we are trying to produce mice deficient in mouse ALDH2 gene.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00099-04 LMBB

Title: ION GRADIENTS AND METABOLIC ENERGY IN ANIMAL TISSUE

Total Staff Years: 2.1

Principal Investigator: Veech RL, MD, PhD (MC, LMBB, NIAAA)

Other Personnel: Crutchfield C (MC, LMBB, NIAAA)
King MT, MS (MC, LMBB, NIAAA)

NIH Collaborators: None

Other Collaborators: Clarke K, PhD (Biochemistry, Oxford University)
Valeri R, MD (Naval Blood Research Laboratory, Boston University)

Sample Type: Neither human subjects nor human tissues

Keywords: metabolism, ion gradients

Summary: The energy of the gradients of the nine major inorganic ions in working perfuse heart are in near equilibrium with each other, the electrical potential between extra and intracellular phase and the DG of ATP hydrolysis (Masuda T et al, J Biol Chem 1990;265:20321-34). The metabolism of ethanol increases the resting electrical potential of hepatocytes from 28 to 40 mV (Veech RL et al, Alcoholism Clin Expt Stud 1994;18:1040-56). We have completed a study of the effects of ethanol-induced change in hepatic voltage upon the gradients of all the amino acids between portal vein blood and liver and the gradients of the 9 major inorganic ions between intracellular and extracellular space in rat liver *in vivo*. To our knowledge, this is the first such study. Our work is also the first to show that the end product of hepatic ethanol metabolism, acetate, completely blocks the gut uptake of l-glutamine. l-Glutamine had been thought to be essential not only for its function, but also for its action as a bacterial barrier. The implication of the ability of acetate to inhibit the gut utilization of l-glutamine remains to be determined. Previously, we showed that merely changing the substrate available altered the DG of ATP hydrolysis in heart (Kashiwaya Y et al, Am J Cardiol 1997;80:50A-64A). Since injuries of any sort induce a stereotypic change in cellular ionic distributions wherein the cell gains Na+, looses K+ and swells, these stereotypic changes of injury can possibly be reversed by simple changes in the compositions of fluids administered to victims of injury or burns. As a result of these studies and our suggestions to a panel convened by the Academy of Medicine, a recommendation has been made that investigation of the feasibility of making new resuscitation fluids be initiated (see: Fluid Resuscitation, state of the science for treating combat casualties and civilian injuries, National Academy Press, 1999). The goal is to improve the standard treatment of hemorrhage and burns, which has not changed over the past 50 years. We are collaborating in this effort.

RESEARCH HIGHLIGHTS: The metabolite of alcohol, acetate, stops the gut uptake of l-glutamine, which is considered essential for maintenance of gut function. The failure of gut to take up l-glutamine during ethanol metabolism may contribute to the malnutrition seen in alcoholics.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Alcohol affects many bodily systems. Here we showed, for the first time, that the major metabolite of ethanol, acetate, prevents the gut from taking up a nutrient, l-glutamine, considered to be essential for the health and proper function of the intestines.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00110-02 LMBB

Title: METABOLIC CONTROL ANALYSIS

Staff Years: 0.1

Principal Investigator: Veech RL, MD, PhD (LMBB, NIAAA)

Other Personnel: None

NIH Collaborators: None

Other Collaborators: Kashiwaya Y, MD (Neurology, Tottori University)
Clarke K, PhD (Biochemistry, Oxford University)

Sample Type: Neither human subjects nor human tissues

Keywords: metabolism, flux control, neurological disease, nutrition

Summary: It is widely believed that the steps in the major metabolic pathways are known and that the control of flux through these pathways occurs at a very limited number of "rate limiting" steps. This concept has led to the design of drugs to alter the kinetics of these "rate limiting enzymes". It has also led to attempts to alter metabolic pathways by altering the amounts of rate limiting enzymes using the techniques of molecular biology. To the dismay of many, such interventions often fail to alter the rates of the pathways under study. These failures have led to an increased awareness that "control" of pathway flux is distributed among many enzymes of a metabolic pathway and can vary from enzyme to enzyme depending upon conditions. Metabolic control theory predicts distribution of control among many enzymes of a pathway (Veech RL & Fell DA, Cell Biochem & Function 1996;14:229-36), however, actual demonstration and testing of such theories was technically difficult. We were the first laboratory to make the required measurements of flux, kinetic and thermodynamic constants of each step, and the levels of all substrates and products required to make such a formal analysis of flux control in a major metabolic pathway (Kashiwaya Y et al, J Biol Chem 1994;269:25502-14). We went on to show that ketone bodies act in heart to overcome insulin resistance in heart (Kashiwaya J et al, Am J Cardiol 1997;80:50A-64A). Since Dr. Kashiwaya left this laboratory, I have continued to collaborate with him. He and others, at the Department of Neurology, have applied the insights from our previous work to investigate the effects of ketone bodies on two neuronal culture models of the two most common degenerative neurological diseases. Alzheimer's disease was modeled by adding amyloid beta 1-42 to embryonic rat hippocampal neuronal cultures and Parkinson's disease was modeled by adding MPP+ to mesencephalic neuronal cultures. In both cases, ketone bodies protected neurons from death induced by these very different toxins. The ability of ketone bodies to protects neurons under these conditions offers the possibility of therapy for these very common diseases as well as other diseases resulting from failure in either glycolysis or mitochondrial energy generation.

RESEARCH HIGHLIGHTS: Control of the flux in a metabolic pathway does not reside in a single "key" enzymatic step but is distributed throughout many steps in the pathway depending upon dietary, hormonal and genetic factors. We have developed a method to quantitatively assign the degree of control exerted at each step. This knowledge allows us to intervene effectively during disorders of metabolic pathways.

SIGNIFICANCE TO BIOMED RESEARCH & PROGRAM: The pathway of glycolysis, the breakdown of sugar, and the Krebs TCA cycle, the combustion of pyruvate to CO2, are the fundamental pathways of energy generation. The two pathways are internally linked. Increased understanding of the metabolic disorder common to many diseases opens the possibility of dietary treatments for common diseases such as Parkinsonism, Alzheimer's disease and trauma.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00235-17 LMBB

Title: NUTRITIONAL EFFECTS ON ESSENTIAL FATTY ACID COMPOSITION

Staff Years: 6.5

Principal Investigator: Salem Jr N, PhD (LMBB, NIAAA)

Other Personnel:
Hibbeln JR, MD (LMBB, NIAAA)
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Greiner RS, PhD (LMBB, NIAAA)
Loewke JD (LMBB, NIAAA)
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Moriguchi T, PhD (LMBB, NIAAA)
Wegher BJ, MS (LMBB, NIAAA)

NIH Collaborators:
Champoux M, PhD (NICHD)
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Other Collaborators:
Hannagan J, PhD (Fetal Alcohol Research Center, Wayne State University)
Hoshiba J, PhD (Animal Center for Medical Res, Okayama Univ Medical School)
Slotnick B, PhD (Department of Psychology, American University)
Sokol R, MD (Fetal Alcohol Research Center, Wayne State University)

Sample Type: Human tissues, fluids, cells, etc.

Keywords: neurosciences, cirrhosis, liver disease, fatty acids, nutritional disorders, prevention, strategies, docosahexaenoic acid, arachidonic acid

Summary: Our past studies as well as those of others have indicated that alcohol abuse leads to a loss in tissue DHA. Nutritional inadequacies, particularly during early development, may also lead to such losses in this essential fatty acid (EFA). In following up this work, it is important to establish what losses in physiological functions are caused by the loss of DHA in various organ systems. Our recent work has focused on the nervous system. A novel application to the field of EFA biology was made with the introduction of olfactory-based learning and memory-related tasks for brain function assessment. This modality was used since Slotnick reported that rats are capable of high level learning of olfactory-based tasks of a nature usually only ascribed to nonhuman primates or higher mammals. Our principal findings are that there is a poorer performance in the acquisition of olfactory set learning in rats where brain and olfactory bulb DHA was lowered through dietary insufficiency. That is, after the rats had acquired the task, they were over-trained in order to determine whether they could achieve the learning set, i.e., make zero or only one mistake in the first twenty trials after an information trial in a two-odor discrimination task. Rats given a safflower oil based diet for two generations did significantly poorer in this regard than did the rats with diets enhanced with oils containing alpha-linolenate and DHA. Spatial maze tasks, including the water maze, also indicated that animals with lower levels of brain DHA performed more poorly, swimming longer and at a higher rate, but finding the platform with a longer latency. In a retention trial, n-3 deficient rats performed significantly worse than the n-3 adequate group, especially when deprived for three generations. Although n-3 deficient rats perform more poorly, it cannot be ascribed to lower activity or motivation as general motor activity was not different between groups and there was no difference in a progressive ratio-licking task in which animals worked for a water reward. The n-3 deficient rats sampled the odors longer than the DHA-adequate animals but still made more subsequent total errors. The n-3 deficient rats were also examined for changes in brain morphology, using quantitative stereologic techniques. Initially, studies focused on hippocampal morphology. After fixing and cresyl violet staining, a variety of features were quantified in rats fed the n-3 deficient or adequate diets for three generations. No statistically significant differences were observed in volume, density or total number of perikarayal neurons in the hippocampus. However, perikarayal size in the septal area of the CA1 field of the hippocampus was greater in the n-3 adequate (containing DHA) group relative to the deficient group. An attempt was made to extend aspects of these studies to nonhuman primates. For the first six months of life, rhesus monkeys were fed either standard infant formula or

formula supplemented with AA and DHA. Striking improvements in visual orientation skills and in motor development were found during the first month of life among infants fed supplemented formulas. Physiological differences in neuronal control of the autonomic nervous system persisted into adolescence. This animal work has important implications for human infant formula composition as formulas are currently devoid of DHA in North America, thus, leading to a decrease in infant brain DHA levels and suboptimal development and function. An effort was begun to develop a method following the work of Junji Hoshiba for auto rearing of rat pups starting from the first day of life. In this way, it will be possible to control the EFA composition of the diet throughout the life cycle of the rat, an animal that is born at a relatively immature stage relative to primates, and has often been used as a model of fetal development. This model will be used to induce changes in brain DHA composition in the first generation of animals and, therefore, may reduce the number of animals and amount of time needed to produce a model of n-3 deficiency. This apparatus will also make possible a new model of fetal alcohol syndrome where animals can be given alcohol from the first days of life. In related work, an effort was made to determine whether changes in essential fatty acids, particularly of the n-3 category, are associated with psychiatric illnesses. Among 200 elderly subjects from rural Iowa, plasma DHA concentrations were lower among depressed women compared to control women. Lower plasma DHA concentrations also predicted greater reports of anxiety and more numerous sleep complaints. Among 11,234 Finnish subjects, reports of infrequent fish consumption predicted more severe scores on the Beck Depression Inventory. Among subjects who recently attempted suicide, low plasma EPA concentrations strongly predicted more severe psychopathology on eight psychiatric rating scales. These scales predict continued risk of suicide. A cross-national comparison of fish consumption and of the prevalence of postpartum depression was conducted in 24 countries. Greater fish consumption predicted a lower risk of incurring postpartum depression ($r = -0.71$, $p<0.0005$). Higher DHA content of mothers' milk also predicted decreased risk of suffering a postpartum depression ($r = -0.82$, $p<0.0006$, $n = 13$).

RESEARCH HIGHLIGHTS: When rats are fed low levels of n-3 fatty acids, their offspring are found to have poor neural development and function along with lower levels of DHA, the principal polyunsaturated fat in brain. Lower n-3 fatty acid status is found in plasma from depressed patients.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: In the United States, we are feeding most of our infants n-3 deficient diets during the crucial period of nervous system development. Therefore, the types of deficits observed in our rodent studies are expected to also occur in the human population.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00262-15 LMBB

Title: DESATURATION OF ESSENTIAL FATTY ACIDS USING STABLE ISOTOPE GC-MS

Staff Years: 1.1

Principal Investigator: Salem Jr N, PhD (LMBB, NIAAA)

Other Personnel: Hibbeln JR, MD (LMBB, NIAAA)
Wegher BJ, MS (LMBB, NIAAA)

NIH Collaborators: None

Other Collaborators: Connor W, MD (Medicine, Oregon Health Sciences University)
Mena P, MD (Pediatrics, INTA University of Chile)
Uauy R, MD (Pediatrics, INTA University of Chile)

Sample Type: Human subjects

Keywords: neurosciences, liver disease, vision/eye/ocular, Retinitis Pigmentosa

Summary: Prior to the recent application of stable isotope based GC/MS methodology, little was known about human essential fatty acid (EFA) metabolism *in vivo*. Our studies have focused on the metabolic capacity of infants in the first week of life and also on human adults. The first phase of this work defined the conversion of linoleic acid to arachidonate and also the conversion of linolenate to docosahexaenoate in infants of varying gestational ages. The somewhat surprising results were that essentially every infant was capable of both n-3 and n-6 fatty acid interconversions *in vivo*. Moreover, there was an inverse relationship of gestational age with plasma deuterium enrichment of DHA, in particular. The least developed infants had the greatest metabolic capability in this respect. This is consistent with the brain growth spurt that occurs in human fetuses during the last trimester. Infants who were small for gestational age had a somewhat diminished metabolic capacity for fatty acids but most of the variance could be explained with gestational age only. In our adult work, normal volunteers, smokers and alcoholic smokers were studied for EFA interconversions *in vivo*. Controlled diet studies indicated that increasing the long chain n-3 fatty acids in the diet led to a decrease in the *in vivo* accretion of the deuterated fatty acid end products in plasma. This is consistent with the well-known phenomenon of end product inhibition. Smokers produced increased amounts and had greater enrichments of deuterated AA and DHA relative to normal non-smokers. Alcoholic smokers had a marked increase in deuterium enrichments of long chain polyunsaturates in plasma, particularly DHA. In alcoholics with liver fibrosis, deuterium enrichment of DHA in liver biopsy samples was also increased relative to alcoholics without liver histopathological findings. These results are significant, as they do not support the commonly held notion in the field that alcohol inhibits elongation/desaturation of EFA. In fact, a hypothesis where alcohol stimulates this pathway would be more consistent with our results. Our hypothesis is that alcohol leads to catabolism of long chain polyunsaturates like DHA. When the alcohol challenge is of sufficient intensity and duration, this will lead to a decrease in the tissue concentration of DHA. Metabolic processes, including elongation/desaturation and transport/acylation, may be increased in the alcoholic in partial compensation for the loss of these important membrane constituents. Our recent studies have examined *in vivo* metabolism of EFA in subjects with Retinitis Pigmentosa. In particular, patients with Ushers II disease or non-Ushers disease were compared to normal volunteers. We observed that the amount or enrichment of deuterated n-3 fatty acid metabolites such as EPA or DHA were significantly increased in Ushers patients whereas there was a decrease relative to normal volunteers in the non-Ushers group. The increased metabolism in the Ushers patients with respect to DHA may be surprising as it has been hypothesized that the retinal concentration of DHA is reduced in Retinitis Pigmentosa and that this may, in part, explain some of the loss in visual function associated with this neurological disease. However, as noted above for alcoholic patients, an increased metabolism may be induced by an increased catabolism that is associated with disease state. These studies point to the need for analysis of increased fatty acid catabolism or indices of lipid peroxidation *in vivo* in these patients. The opposite direction of response in the non-Ushers patients points to a quite distinct etiology of this disease.

RESEARCH HIGHLIGHTS: Infant Study—Our studies of newborn infant metabolism indices a well-developed EFA metabolic capacity in the first week of life. The rates of metabolism indicate that infants require preformed sources of DHA in their diets.

We also performed the first metabolic studies on fatty acids in patients with two forms of Retinitis Pigmentosa and found metabolic abnormalities.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: A new paradigm is emerging from this work suggesting that organ damage associated with alcoholism (and other diseases) is potentiated by a diet which is inadequate in EFA, particularly the n-3 fats.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00284-10 LMBB

Title: ALTERATIONS IN LIPID METABOLISM IN THE NERVOUS SYSTEM BY ETHANOL

Staff Years: 5.1

Principal Investigator: Kim H, PhD (MS, LMBB, NIAAA)

Other Personnel: Akbar M, PhD (MS, LMBB, NIAAA)
Hamilton J, PhD (MS, LMBB, NIAAA)
Kevala K, MS (MS, LMBB, NIAAA)
Lau A, BS (MS, LMBB, NIAAA)
Li B, PhD (MS, LMBB, NIAAA)

NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: docosahexaenoic acid, apoptosis, neuronal cells, phosphatidylserine, melatonin, ethanol, PLA2, lipoxygenase, pineal, mass spectrometry

Summary: The principal objective of this study is to elucidate metabolic and biologic functions of polyunsaturated fatty acids, docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) in the nervous system with particular reference to their modulation by ethanol. Previously, we found that 22:6n-3 promotes the accumulation of phosphatidylserine (PS), which is thought to be involved in growth factor signaling, leading to cell survival. During this period, the effect of polyunsaturates on the survival of neuronal cells was further investigated along with the underlying mechanisms of this effect. The results obtained by the differential sedimentation assay, DNA ladder formation and Hoechst staining, as well as caspase-3 activity assay consistently indicated that 22:6n-3 prevented the apoptotic cell death of both Neuro 2A and PC-12 cells, but only after a prolonged period of enrichment. Phospholipid molecular species analysis by electrospray LC/MS revealed that the extent of protective effect correlated with the incorporation of 22:6n-3 into PS, suggesting that 22:6n-3 as a membrane phospholipid constituent, especially as PS, may be important for the protective effect. According to *in vitro* biomolecular interaction analysis, the interaction between unilamellar vesicles of phospholipids and Raf-1 kinase required the presence of PS in the vesicle and the extent of interaction was indeed dependent on the PS composition. These results appear to support the view that PS accumulation promoted by 22:6n-3 may be important in growth factor signaling. We also found that melatonin, the major product of the pineal gland, regulates polyunsaturated fatty acid metabolism at both PLA2 and 12-lipoxygenase levels, suggesting a role of polyunsaturated fatty acids in biochemical functions involving melatonin.

RESEARCH HIGHLIGHTS: Docosahexaenoic acid (a long chain polyunsaturated n-3 fatty acid) is protective for the survival of neuronal cells.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Docosahexaenoic acid in the central nervous system can be modulated by ethanol. Therefore, the information regarding the protective effect of docosahexaenoic acid would be helpful for situations where chronic ethanol resulted in neuronal degeneration.

FY 1999 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1998 - 30 SEPTEMBER 1999)

LABORATORY OF MOLECULAR & CELLULAR NEUROBIOLOGY

FORREST F. WEIGHT, M.D., CHIEF

DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE & ALCOHOLISM
NATIONAL INSTITUTES OF HEALTH

SYNOPSIS
LABORATORY OF MOLECULAR & CELLULAR NEUROBIOLOGY
1 OCTOBER 1998 - 30 SEPTEMBER 1999

INTRODUCTION

In recent years, great progress has been made in understanding the function of the central nervous system (CNS) at the cellular and molecular level. The Laboratory of Molecular & Cellular Neurobiology (LMCN) was established in Fiscal Year 1992 to utilize this increased knowledge of neurobiology to investigate the cellular and molecular mechanisms of alcohol actions in the nervous system. These investigations are intended to provide a foundation for understanding the cellular and molecular basis of alcohols' behavioral effects, such as intoxication, anxiolytic actions and anesthesia, as well as pathophysiologic phenomena, such as the neurotoxicity of alcohol that results in cerebral atrophy and alcohol-induced alterations in neural development that are manifested as the fetal alcohol syndrome.

Administratively, LMCN has three sections: the Section on Physiology, the Section on Pharmacology, and the Section on Molecular Neuroscience. Dr. Forrest Weight is Chief, Section on Physiology, and Acting Chief of the other Sections. Dr. Robert Peoples was appointed tenure-track investigator in February 1998; he is Acting Chief, Unit on Cellular Neuropharmacology, in the Section on Pharmacology. On the recommendation of the NIAAA Board of Scientific Counselors in 1998, a nationwide search for a tenure-track cellular neurophysiologist was carried out in Fiscal Year 1999; however, an appointment was not made, as the search committee did not reach a consensus on the candidates. Because the NIAAA Board of Scientific Counselors also recommended recruitment of a tenure-track and/or tenured molecular neurobiologist, a combined search for a cellular neurophysiologist and/or molecular neurobiologist is planned in Fiscal Year 2000.

The Section on Physiology investigated the neuronal actions of alcohol and provided further evidence that neurotransmitter receptors are cellular sites of alcohol action in the nervous system. This evidence is an important advance in alcohol research, as it permits detailed investigation of the cellular and molecular mechanisms of alcohol actions at these sites. Moreover, since neurotransmitter receptors mediate communication between neurons at synapses, the demonstration that alcohol can affect the function of these receptors suggests that these actions may underlie some of the behavioral effects of alcohol. In addition, studies are in progress on the physiological regulation of the alcohol sensitivity of neurotransmitter receptors. These studies suggest that such physiological approaches will advance our knowledge of the cellular basis of alcohol actions in the nervous system.

In the Section on Pharmacology, Dr. Peoples investigated the pharmacology of alcohol actions in the nervous system. His studies have provided evidence that alcohols affect the function of NMDA receptors by acting on an extracellular site, and suggest that "cutoff effect" for NMDA receptors is due to the insolubility of long-chain alcohols, rather than to a size exclusion mechanism.

The Section on Molecular Neuroscience used molecular biological approaches to investigate the molecular basis of alcohol action on neurotransmitter receptors. Studies in this Section have provided evidence that the alcohol sensitivity of neurotransmitter receptors is determined by the molecular structure of the receptor. In addition, studies are in progress on the molecular regulation of alcohol sensitivity of neurotransmitter receptors. These studies hold the promise that such molecular biological approaches will advance our knowledge of the molecular basis of alcohol actions in the nervous system.

SECTION ON PHYSIOLOGY

Research studies in the Section on Physiology are directed toward elucidating the cellular mechanisms of alcohol actions in the nervous system. The behavioral effects of alcohol are well known; however, the mechanisms by which alcohol produces those effects have not been established. The investigations in this Section use primarily electrophysiological methods, such as the patch-clamp technique, to study the function of membrane receptors and ion channels in neurons and neural cell-lines, and the actions of alcohol on those membrane proteins.

In experiments on voltage-gated membrane ion channels in adult mammalian neurons, these ion channels, which underlie the intrinsic electrical excitability of neurons, were found to be insensitive to intoxicating and anesthetic concentrations of ethanol (10 -100 mM). By contrast, a number of different types of neuronal neurotransmitter-gated membrane ion channels were found to be sensitive to this range of ethanol concentrations. This type of neurotransmitter receptor mediates fast excitatory or inhibitory synaptic transmission between neurons in the CNS. The effects of ethanol on these ion channels are summarized briefly below.

Excitatory Amino Acid-Activated Channels: Glutamate is the major excitatory neurotransmitter in the mammalian CNS. Glutamate activates at least three types of neurotransmitter-gated membrane ion channels, designated by their responses to the agonists, NMDA, AMPA and kainate.

NMDA receptor-channels mediate a slower component of synaptic excitation thought to be involved in several types of important neural phenomena including cognitive function, motor control, synaptic plasticity and certain types of learning. In hippocampal neurons, ethanol was found to inhibit the function of NMDA receptors in a concentration-dependent manner in a concentration range associated with intoxication. In addition, the potency of several short-chain alcohols for inhibiting NMDA receptor function were found to be correlated with their intoxicating potency, suggesting that ethanol-induced inhibition of NMDA receptor function may contribute to the neural and cognitive impairments associated with intoxication. Investigation of the mechanism involved in the inhibition of NMDA receptor function by ethanol indicates that ethanol inhibits NMDA receptor function by a non-competitive mechanism that does not involve block of the ion channel, alteration of the ion selectivity of the channel or interaction with a number of regulatory sites on the receptor. Single-channel experiments indicate that ethanol inhibits NMDA receptor function by altering channel gating. Straight-chain alcohols inhibit NMDA receptor function with increasing potency as the carbon chain-length is increased up to 8 carbon atoms; however, alcohols with more than 8 carbon atoms do not affect NMDA receptor function. This "cutoff" for inhibition of NMDA receptor function by a series of straight-chain alcohols is similar to cutoffs reported previously for two behavioral indices of intoxication, ataxia and loss-of-righting reflex, suggesting that alcohol inhibition of NMDA receptor function may contribute to the behavioral manifestations of alcohol intoxication.

AMPA- and kainate-activated channels (non-NMDA glutamate receptor-channels) mediate fast transmission at most excitatory synapses in the CNS. In concentrations less than 50 mM, ethanol has relatively little effect on the function of non-NMDA glutamate receptor-channels in neurons. In concentrations greater than 50 mM, however, ethanol produces a concentration-dependent inhibition of these receptors. Blood-ethanol concentrations from 50-100 mM are associated with the signs of general anesthesia. Since ethanol inhibits the function of non-NMDA glutamate receptor-channels over this concentration range, and these channels mediate fast synaptic transmission at most excitatory synapses in the CNS, it seems possible that the ethanol inhibition of these receptors may contribute to the general anesthetic effects of ethanol. This hypothesis is supported by the observations that the general anesthetic agents, trichloroethanol (the active metabolite of chloral hydrate), barbiturates and volatile general anesthetics, all inhibit the function of non-NMDA glutamate receptor-channels in an anesthetic concentration range.

Inhibitory Amino Acid-Activated Channels: The major inhibitory neurotransmitter in the brain is gamma-aminobutyric acid (GABA). Some laboratories have reported that GABA-gated membrane ion channels (GABA-A receptors) are sensitive to pharmacologic concentrations of ethanol (10 - 100 mM), whereas other labs have reported that they are not. It has been proposed that the reason for these divergent observations is that protein kinase C (PKC)-induced phosphorylation of the gamma-2L subunit is required for the receptor to be sensitive to ethanol. This hypothesis was studied on GABA-A receptor-mediated responses in rat dorsal root ganglion (DRG) neurons. The presence of the gamma-2L subunit was confirmed using RT-PCR. GABA responses were found to remain insensitive to ethanol concentrations ranging from 2.5 to 100 mM despite: extracellular application of the PKC-activator, phorbol ester; raising intracellular $Ca2+$ to activate PKC; intracellular application of PKC; and, extracellular application of phorbol ester plus intracellular application of protein phosphatase inhibitors to prevent breakdown of PKC. The observations do not support the hypothesis that PKC phosphorylation of the gamma-2L subunit induces ethanol sensitivity of GABA-A receptors. The alcohol sensitivity of GABA-A receptors was also studied in mouse hippocampal neurons. Straight-chain alcohols from ethanol to dodecanol enhanced GABA responses, whereas higher alcohols had no effect. The EC50 for ethanol was 2,160 mM (approximately 20 times the lethal concentration in non-tolerant humans). The n-alcohol

potency curve for enhancement of GABA-A receptor function was similar to the n-alcohol potency curve for anesthesia in tadpoles. In addition, the anesthetic potency of n-alcohols in rats more closely reflected NMDA receptor modulatory potency for short-chain alcohols and GABA-A receptor modulatory potency for long-chain alcohols.

Serotonin-Activated Channels: It has been suggested that serotonin type 3 (5HT3) receptors may be involved in alcohol's reward mechanisms. In neurons and neural cell-lines, ethanol was found to potentate 5HT3 receptor-mediated responses, at low agonist concentrations, in a concentration-dependent manner over the concentration range 10 - 100 mM. However, the potentation by ethanol decreases with increasing serotonin concentration, suggesting that ethanol may increase the potency of serotonin action. Investigations are currently in progress to determine the mechanism involved in this ethanol-induced potentation of 5HT3 receptor-mediated responses. In addition, a "cutoff effect" for a series of straight chain alcohols has been observed for 5HT3 receptor-mediated responses; however, it appears that the cutoff may be affected by agonist concentration, so that experiments are in progress to determine the nature of the cutoff phenomenon for 5HT3 receptors.

ATP-Activated Channels: Extracellular ATP (adenosine 5'-triphosphate) has been found to function as an excitatory neurotransmitter and to gate membrane ion channels in neurons, in both the central and peripheral nervous systems. There appear to be several types of ATP-gated ion channels in neurons, based on molecular biological cloning, as well as physiologic and pharmacologic properties of the receptors. Ethanol has been found to inhibit the function of one type of ATP-gated channel over a pharmacologic concentration range with an IC50 value of 68 mM. Methanol is less potent and 1-propanol is more potent in inhibiting the ATP-activated current; however, 1-butanol and 1-pentanol are without effect on this current. In addition, the intracellular application of 100 mM ethanol does not affect the inhibition by extracellularly applied ethanol, by the intracellular application of activators or inhibitors of G-proteins or by activators or inhibitors of protein kinase A (PKA) or protein kinase C (PKC). The mechanism of ethanol inhibition of ATP-gated channels differs from the mechanism of ethanol inhibition of NMDA receptors. For NMDA receptors, ethanol decreases maximal response (Emax) of the agonist concentration-response curve without affecting the EC50, whereas for ATP-gated channels, ethanol shifts the ATP concentration-response curve to the right in a parallel manner, increasing the EC50 for ATP without altering its Emax. Thus, the effect of ethanol on the function of ATP-gated channels appears to be a competitive type of inhibition; however, an ethanol-induced decrease in the affinity of the agonist binding site would also result in a parallel shift to the right of the agonist concentration-response curve. To distinguish between these mechanisms, the effect of ethanol was studied on the activation and deactivation kinetics of ATP-activated current. Ethanol decreased the time-constant of deactivation of ATP-activated current, without affecting the time-constant of activation. The observations suggest that ethanol does not competitively antagonize ATP activation of the receptor, but rather ethanol has an allosteric action to decrease the affinity of the ATP binding site on the receptor.

Acetylcholine-Activated Channels: The muscle-type nicotinic acetylcholine (nACh) receptor is the most extensively studied and characterized neurotransmitter-gated membrane ion channel. However, the effect of ethanol on the function of these receptor-channels has not been well characterized with modern electrophysiological techniques. Therefore, the action of ethanol has been studied on this receptor in order to gain insight into the structure-activity relationships of ethanol action. In studies on mouse nACh receptors containing alpha-beta-delta-epsilon subunits, ethanol concentrations from 10-150 mM produce a concentration dependent potentation of currents activated by low concentrations of ACh. Associated with the ethanol-induced potentation is an increased desensitization rate of the current. However, with ACh concentrations greater than 25 mM, ethanol reduces peak current amplitude, presumably due to the rapid onset of desensitization, as occurs with high agonist concentrations. Investigations on the mechanisms involved in these ethanol actions are currently in progress.

Summary and Conclusions: The studies in the Section on Physiology have provided evidence that neurotransmitter-gated membrane ion channels are cellular sites of alcohol action in the nervous system. These studies have also shown that alcohol effects on the function of different types of neurotransmitter-gated membrane ion channels can involve different specific mechanisms. This is in contrast to the belief, for over 90 years, that alcohol exerts its effects in the nervous system through a non-specific action on membrane lipids. These studies have also found that a series of straight-chain alcohols exhibits a potency cutoff for affecting the function of all of the neurotransmitter-gated channels that have been tested. In addition, the cutoff has been found to be different for each receptor type that has been studied, suggesting that the molecular determinants of the cutoff effect may be different for different types of

receptors. In addition, studies are in progress on the physiological regulation of alcohol sensitivity of these receptors. The progress that has been made in these studies suggests that such physiological approaches will advance our knowledge of the cellular basis of alcohol action in the nervous system.

SECTION ON PHARMACOLOGY

The research in the Section on Pharmacology is directed toward elucidating the pharmacology of alcohol actions in the nervous system. As indicated above, previous studies had found that straight-chain alcohols with 8 or fewer carbon atoms inhibit NMDA receptor function, whereas larger straight chain alcohols do not affect the function of NMDA receptors. This "cutoff effect" was originally proposed to result from size exclusion; i.e. the alcohols act by binding in a hydrophobic pocket of circumscribed dimensions, so that alcohols that are larger than the size of this pocket are unable to bind in the pocket and therefore have no effect. To test this hypothesis, Dr. Peoples tested the effect on NMDA receptor function of a series of straight-chain 1,omega-diols, which have lower hydrophobicity but slightly greater molecular volume than the corresponding straight-chain alcohols. He found that diols with 9 or 10 carbon atoms were able to inhibit NMDA receptor function, despite having molecular volumes greater than the corresponding straight-chain alcohols. This suggests that the cutoff effect for NMDA receptors most probably result from the inability of long-chain alcohols to achieve adequate concentrations at the site of alcohol action due to low aqueous solubility, rather than from a size-exclusion mechanism. Dr. Peoples also performed experiments to identify the location of the site of alcohol action on NMDA receptors. He found that the intracellular application of 1-pentanol did not inhibit NMDA receptor function, nor did it alter the inhibitory effect of extracellularly applied ethanol or pentanol. In addition, the application of ethanol to the cytoplasmic face of inside-out membrane patches did not alter NMDA-activated current. The inhibitory effect of extracellularly applied ethanol was also not altered by truncation of the intracellular C-termini of the NR1/NR2B subunits of the NMDA receptor. The results suggest that the action of alcohols involve an extracellular domain of the NMDA receptor.

SECTION ON MOLECULAR NEUROSCIENCE

The research activities in the Section on Molecular Neuroscience are directed toward understanding alcohol actions in the nervous system at the molecular level. This Section uses a combination of molecular biological and electrophysiological research methods to address these questions. In the studies carried out in the Section, alcohol effects have been studied on the physiology and pharmacology of recombinant neurotransmitter receptors using primarily *Xenopus* oocytes as an expression system. Those studies are summarized briefly below.

Recombinant NMDA Receptors: Although ethanol inhibits NMDA receptor function in a number of regions of the nervous system, the sensitivity of NMDA receptors to ethanol has been found to be different in different brain regions and in different types of neurons. Cloning studies have revealed a molecular diversity of NMDA receptors and *in situ* hybridization indicates a differential distribution of different subunits throughout the brain, suggesting that differences in NMDA receptor subunit composition might be responsible for the differences in NMDA receptor sensitivity to ethanol in different types of neurons. Therefore, the ethanol sensitivity of different NMDA receptor subunits was studied using recombinant NMDA receptor subunits expressed in *Xenopus* oocytes. Various NMDA receptor subunit combinations were found to exhibit a differential sensitivity to ethanol. The mouse heteromeric subunit combinations epsilon-1/zeta-1 and epsilon-2/zeta-1 are inhibited by 50 mM ethanol, whereas the heteromeric combination epsilon-3/zeta-1 and the homomeric zeta-1 subunits are not significantly affected by this concentration of ethanol. The sensitivity of the epsilon-4/zeta-1 subunit combination is similar to that of the epsilon-3/zeta-1 subunit combination. In addition, there are differences in the ethanol concentration-response curves for different subunit combinations. These observations are consistent with the possibility that NMDA receptor subunit composition may contribute to differences in the ethanol sensitivity of NMDA receptors observed in different brain regions and in different types of neurons.

Recombinant Non-NMDA Glutamate Receptors: The ethanol sensitivity of non-NMDA glutamate receptor-channels was studied using recombinant non-NMDA glutamate receptors types 1-3 (GluR1-3) expressed in *Xenopus* oocytes. Ethanol was found to inhibit the function of these receptors in a concentration-dependent manner over the concentration range 50-500 mM. The ethanol inhibition of the responses of the GluR1+2+3 heteromeric combination has an IC50 value of 176 mM, and the IC50 value

for inhibition of the GluR3 subunit is 212 mM. These values are in a similar range to the ethanol sensitivity observed for non-NMDA glutamate receptor-channels in neurons. In addition, for a series of straight-chain alcohols from methanol to heptanol, the potency for inhibition of GluR receptor-mediated responses increases in proportion to the chain-length of the alcohol. However, despite increased hydrophobicity, a distinct cutoff for the inhibition of these receptors is observed for alcohols with more than 7 carbon atoms for both GluR1 and GluR3 receptors. Since these two subunits have considerable homology in molecular structure, the similar cutoffs for these subunits may indicate that the molecular basis of the cutoff effect may be similar for GluR1 and GluR3.

Recombinant and Expressed GABA-A Receptors: It has been reported that GABA-A receptors from long-sleep (LS) mice are more sensitive to ethanol than GABA-A receptors from short-sleep (SS) mice, and it has been proposed that the ethanol sensitivity is due to the presence of the gamma-2L subunit of the GABA-A receptor, based on studies in *Xenopus* oocytes expressing LS or SS mouse brain mRNA, or the cRNA of mouse GABA-A receptors containing alpha-1/beta-1/gamma-2L subunits. However, we have been unable to repeat the observations on which those conclusions were based, *viz.* we have been unable to find effects of ethanol, in concentrations from 10 - 100 mM, on GABA-A receptor-mediated responses in *Xenopus* oocytes expressing LS or SS mouse brain mRNA, or mouse GABA-A receptors containing alpha-1/beta-1/gamma-2L subunits. Our inability to confirm the observations on which the gamma-2L hypothesis is based raises questions about the molecular determinants of GABA-A receptor sensitivity to ethanol. Experiments are currently in progress attempting to elucidate these questions.

Recombinant nACh Receptors: The alpha7 subtype of nACh receptors is one of the most abundant nicotinic receptors in the mammalian CNS, and it has been suggested that it may be important in the addiction to nicotine. In studies on the effect of ethanol on recombinant nACh-alpha7 receptors expressed in *Xenopus* oocytes, it was surprising to find that ethanol inhibits the function of this receptor, because ethanol potentates the function of the muscle-type nACh receptor (see above). The inhibition of nACh-alpha7 receptor function by ethanol is of the non-competitive type, *viz.* it decreases Emax without affecting the EC50 of the agonist concentration-response curve, which is similar to the ethanol inhibition of NMDA and non-NMDA glutamate receptor function, but it differs from the response of ATP-gated channels, where ethanol increases the EC50 without affecting Emax. In addition, investigation of recombinant muscle-type nACh receptors suggests that the epsilon subunit is required for ethanol action on that receptor, because ethanol does not affect the function of nACh receptors composed of alpha-beta-delta subunits.

Recombinant 5HT3 Receptors: As noted above, studies in the Section on Physiology have shown that ethanol can potentiate 5HT3 receptor function at low agonist concentrations in neurons and neural cell lines. However, it was also found that 5HT3 receptors are insensitive to ethanol in approximately 15% to 25% of these cells. Since the 5HT3 receptor has been cloned, the receptor was expressed in *Xenopus* oocytes to study the molecular determinants of ethanol sensitivity. For the mouse recombinant 5HT3 receptor, it was found that ethanol potentiates the response activated by low concentrations of 5HT in all of the cells studied. Since the intracellular loop of the 5HT3 receptor has consensus sequences for phosphorylation, and PKC activation potentiates the response of these receptors, experiments are in progress, using molecular biological methods such as site-directed mutagenesis and deletion mutation, to determine whether these consensus sequences for phosphorylation can regulate the sensitivity of 5HT3 receptors to ethanol. It has also been found that single amino acid mutations in the N-terminal domain of the 5HT3 receptor can alter the ethanol sensitivity of the receptor. Experiments are currently in progress to determine the mechanism involved in this alteration of ethanol sensitivity.

Chimeric Nicotinic-Serotonergic Receptors: Chimeric proteins have been extremely valuable for determining relationships between structural domains and functional properties of membrane proteins. One such construct, a chimeric receptor from two different neurotransmitter-gated membrane ion channels, with the N-terminal domain from the nACh-alpha7 receptor and the transmembrane and C-terminal domains from the 5HT3 receptor, manifests activation by nicotinic agonists but channel specificities of the 5HT3 receptor. Since ethanol and the volatile anesthetics, halothane and isoflurane, inhibit the function of nACh-alpha7 receptors and potentiate the function of 5HT3 receptors, this chimeric nicotinic-serotonergic receptor was used to study whether the modulatory actions of ethanol and volatile anesthetics are associated with the N-terminal or the transmembrane and C-terminal domains of the receptor. Both ethanol and the volatile anesthetics were found to inhibit the response of the chimeric receptor in a manner similar to that of the nACh-alpha7 receptor, suggesting that the alcohol and anesthetic actions involve the N-terminal domain of the receptor.

Summary and Conclusions: The studies in the Section on Molecular Neuroscience have provided evidence that the alcohol sensitivity of neurotransmitter-gated membrane ion channels can be determined by the molecular structure of the receptor. In addition, studies are in progress on the molecular regulation of alcohol sensitivity of these receptors. These studies hold the promise that such molecular biological approaches will advance our knowledge of the molecular basis of alcohol actions in the nervous system.

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Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00007-07 LMCN

Title: MOLECULAR NEUROBIOLOGY AND ALCOHOL ACTIONS

Staff Years: 3.2

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Sample Type: Neither human subjects nor human tissues

Keywords: neuroscience, alcohol, receptor, ion channel, oocyte, molecular biology, electrophysiology

Summary: The molecular basis of alcohol action in the nervous system is poorly understood. Neurotransmitter receptors have been found to be alcohol-sensitive membrane proteins. We studied molecular determinants of alcohol sensitivity of these proteins. A nicotinic-serotonergic chimera indicated that the N-terminal domain is involved in the action of ethanol on this protein (Mol Pharmacol 1996;50:1010). We found that site-directed mutagenesis of single amino acids in the N-terminal domain of the 5HT3 receptor alter the ethanol sensitivity of this protein. The wild type and mutant receptors were expressed in Xenopus oocytes and their function studied using two-electrode voltage-clamp. Substitution of arginine (R) for the 245 alanine (A) [R245A] greatly increased the ethanol sensitivity of the receptor. On the other hand, substitution arginine (R) for the 243 alanine (A) [R243A] decreased ethanol sensitivity of the receptor. The increased ethanol sensitivity of R245A appears to be associated with an increase in the agonist affinity of the receptor, whereas the decreased ethanol sensitivity of R243A appears to be associated with a decrease in the agonist affinity of the receptor. Experiments are currently in progress using different amino substitutions to study whether there also is an association of ethanol sensitivity with the hydrophobicity, charge or molecular volume of the substituted amino acids. Observations indicate that single amino acids in the molecular structure of a receptor can affect ethanol sensitivity of the receptor.

RESEARCH HIGHLIGHTS: This project investigates the molecular basis of alcohol actions in the nervous system using molecular biological techniques. These studies have provided evidence that the alcohol sensitivity of some types of neurotransmitter receptors is determined by the molecular structure of the receptor. In addition, studies are in progress on the molecular regulation of alcohol sensitivity of neurotransmitter receptors.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The studies hold the promise that such molecular biological approaches will advance our knowledge of the molecular mechanisms of alcohol action in the nervous system and provide a foundation for understanding the molecular basis of alcohol abuse and alcoholism.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00448-02 LMCN

Title: CELLULAR NEUROPHARMACOLOGY AND ALCOHOL ACTIONS

Staff Years: 1.2

Principal Investigator: Peoples RW, PhD (P, LMCN, NIAAA)

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NIH Collaborators: None

Other Collaborators: None

Sample Type: Neither human subjects nor human tissues

Keywords: alcohol, ion channel, glutamate, NMDA, patch-clamp, primary culture, central nervous system, transfection

Summary: Neurotransmitter-gated membrane ion channels are among the most important target sites of alcohol action in the nervous system, although the manner in which alcohols modulate the function of these transmembrane proteins has not been established. The aim of this project is to investigate the actions of alcohols and related compounds on neurotransmitter-gated ion channels thought to be involved in producing the intoxicating effects of alcohols in nervous tissue. Previous studies have shown that function of the N-methyl-D-aspartate (NMDA) receptor-channel, a type of receptor for excitatory neurotransmitter glutamate, is inhibited by intoxicating concentrations of ethanol. In addition, n-alcohols with 8 or fewer carbon atoms inhibit NMDA receptor-ion channel function, while larger n-alcohols do not. This "cutoff effect" was originally proposed to result from size-exclusion; i.e., alcohols act by binding to an amphiphilic region on the receptor-ion channel protein, and alcohols above the cutoff are unable to bind because their size exceeds the dimensions of the site. To test this hypothesis, the effects of a series of straight-chain 1,omega-diols, which have lower hydrophobicity but slightly greater molecular volume than the corresponding alcohols, were evaluated on NMDA-activated current in mammalian cells transfected with NR1/NR2B NMDA receptor subunits. These experiments revealed that diols with 9 or more carbon atoms were able to inhibit NMDA-activated current, despite having molecular volumes greater than those of the corresponding alcohols. This suggests that the cutoff effect on NMDA receptor-ion channels most probably result from the inability of long-chain alcohols to achieve adequate concentrations at the site of action on the protein due to low aqueous solubility, rather than from a size-exclusion mechanism. Experiments were also performed to identify the location of the site of alcohol action on the NMDA receptor in mammalian cells transfected with NR1/NR2B NMDA receptor subunits. Intracellular application of 1-pentanol did not inhibit NMDA-activated current, and did not alter the inhibitory effect of extracellularly applied ethanol or pentanol. Application of ethanol to the cytoplasmic face of inside-out membrane patches did not alter NMDA-activated current. In addition, the inhibitory effect of extracellularly applied ethanol was not altered by truncation of the intracellular C-termini of both subunits. These results are consistent with an action of alcohols on the extracellular domain of the NMDA receptor-ion channel.

RESEARCH HIGHLIGHTS: The *N*-methyl-D-aspartate (NMDA) receptor is a protein that plays an important role in producing the intoxicating effects of alcohol in the brain. Although this protein is exposed on both the inner and outer surfaces of cells in the brain, deletion of most of the protein that is exposed to the inside of the cell did not change its sensitivity to alcohol. In addition, NMDA receptors in isolated membrane patches were not inhibited by alcohol applied to the intracellular side. These findings agree well with previous results from this unit, and establish that the site of alcohol on the NMDA receptor is on a region of the protein that is exposed to the outside of the cell.

Recent results from this unit have also clarified the mechanism responsible for the "cutoff" effect (loss of activity by alcohols greater than a certain size) on the NMDA receptor. A number of diols, which are similar to alcohols but have higher water solubility, were able to inhibit NMDA receptors despite being

much larger than the molecular size cutoff for alcohols. This result suggests that the "cutoff" effect of long-chain alcohols on NMDA receptors is due to their low aqueous solubility, rather than their large molecular size.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Because the NMDA receptor is thought to be among the most important sites in the brain through which alcohol produces its intoxicating effects, determining the site and mechanism of action of alcohol on this receptor are of great importance. These studies constitute important steps toward this goal, as well as toward the larger goal of understanding the molecular basis of alcohol intoxication.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00479-16 LMCN

Title: SYNAPTIC MECHANISMS AND ALCOHOL ACTIONS

Staff Years: 3.1

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Sample Type: Neither human subjects nor human tissues

Keywords: neuroscience, alcohol, neuron, synapse, receptor, ion channel, electrophysiology

Summary: The cellular basis of alcohol action in the nervous system is poorly understood. Alcohols can affect the function of neurotransmitter receptors; however, the mechanisms have not been established. We used patch-clamp recording to study the cellular mechanisms of alcohol action on these receptors. Some laboratories have reported that GABAa receptors are sensitive to pharmacologic concentrations of ethanol (5-100 mM), whereas others have reported that they are not. It has been proposed that the reason for these divergent observations is that PKC-induced phosphorylation of the gamma-2-L subunit is required for the receptor to be sensitive to ethanol. We studied this hypothesis in rat dorsal root ganglion neurons. The presence of the gamma-2-L subunit mRNA was confirmed using RT-PCR. GABA responses remained insensitive to ethanol concentrations from 2.5-100 mM despite: (i) extracellular phorbol ester; (ii) raising intracellular Ca2+; (iii) intracellular PKC; and (iv) extracellular phorbol ester plus intracellular protein phosphatase inhibitors. The observations do not support the hypothesis that PKC phosphorylation of gamma-2-L induces ethanol sensitivity of GABAa receptors. We also studied the alcohol sensitivity of GABAa receptors in mouse hippocampal neurons. N-alcohols from ethanol to dodecanol enhanced GABA responses, whereas higher alcohols had no effect. The EC50 for ethanol was 2160 mM. The n-alcohol potency curve for enhancement of GABAa receptor function was similar to the n-alcohol potency curve for anesthesia in tadpoles. In addition, the anesthetic potency of n-alcohols in rats more closely reflected NMDA receptor modulatory potency for lower alcohols and GABAa receptor modulatory potency for higher alcohols.

RESEARCH HIGHLIGHTS: This project investigates alcohol actions on neuronal neurotransmitter receptors using electrophysiological techniques. These studies have provided evidence that neurotransmitter receptors are cellular sites of alcohol action in the nervous system. These studies have also demonstrated the mechanisms by which alcohol affects the function of different types of neurotransmitter receptors. In addition, studies are in progress on the physiological regulation of alcohol sensitivity of neurotransmitter receptors.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The studies suggest that such physiological approaches will advance our knowledge of the cellular mechanisms of alcohol action in the nervous system and provide a foundation for understanding the cellular basis of alcohol abuse and alcoholism.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00480-16 LMCN

Title: NERVE CELL EXCITABILITY AND ALCOHOL ACTIONS

Staff Years: 0.4

Principal Investigator: Weight FF, MD (P, LMCN, NIAAA)

Other Personnel: Stewart RR, PhD (P, LMCN, NIAAA)

NIH Collaborators: None

Other Collaborators: Luskin MB, PhD (Emory University School of Medicine)
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Sample Type: Neither human subjects nor human tissues

Keywords: neuroscience, alcohol, neuron, excitability, ion channel, electrophysiology

Summary: Alcohol is classified pharmacologically as a central nervous system depressant. The cellular mechanisms that underlie this alcohol-induced depression of nervous system excitability, however, are poorly understood. This project investigated voltage dependent, membrane ion channels in neuronal precursor cells. Precursor cells from the anterior subventricular zone (SVZa) of neonatal rat forebrain express neuron-specific markers and divide while migrating along the path to the olfactory bulb, where they differentiate into granule and periglomerular interneurons. SVZa cells also express neuron specific tublin and divide *in vitro.* Voltage dependent potassium currents were studied using whole-cell patch-clamp recording in SVZa cells isolated from newborn rats and cultured for 1 day. A-type potassium current (I-K[A]) was recorded in the presence of 300 nM tetrodotoxin and 20 mM tetraethylammonium (TEA); it was identified by its properties of steady-state half-inactivation (-90 mV) and rapid recovery from inactivation (20 ms at -130 mV). Inactivation of I-K[A] was voltage-independent and had a time-constant of 15 ms. The second potassium current identified resembled a delayed rectifier (I-K[DR]) by inactivating slowly over several seconds and being blocked reversibly by external TEA (IC50 4.1 mM). I-K[DR] exhibited steady-state half-inactivation of -50 mV and sigmoidal activation kinetics, with time-constants ranging from 11 ms at -40 mV to 1.5 ms at 100 mV. Although SVZa cells undergo division in culture, their properties resemble those of postmitotic cerebellar granule neurons. Future experiments are planned to study the effect of alcohol on these currents.

RESEARCH HIGHLIGHTS: This project investigates alcohol actions on neuronal voltage-gated membrane ion channels using electrophysiological techniques. These studies have indicated that in most cases these channels, which underlie the intrinsic electrical excitability of neurons, are relatively insensitive to intoxicating and anesthetic concentrations of alcohol.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The studies suggest that such physiological approaches will advance our knowledge of the cellular mechanisms of alcohol action in the nervous system and provide a foundation for understanding the cellular basis of alcohol abuse and alcoholism.

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FY 1999 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1998 - 30 SEPTEMBER 1999)

LABORATORY OF NEUROGENETICS

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DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE & ALCOHOLISM
NATIONAL INSTITUTES OF HEALTH

SYNOPSIS
LABORATORY OF NEUROGENETICS
1 OCTOBER 1998 - 30 SEPTEMBER 1999

INTRODUCTION

The primary mission of this laboratory is to identify vulnerability and protective alleles that underlie alcoholism's measured heritability. Identification of these alleles will lead to better understanding of mechanisms of vulnerability, individualization of treatment and definition of gene-environment interactions. Two complementary approaches, whole genome linkage analysis and direct scanning of candidate genes, are being used. Examples of these approaches are a whole genome linkage scan of a Southwestern Indian tribe and discoveries of functionally significant sequence variation within genes expressed in brain. The whole genome linkage approach enables detection of unknown genes of moderate or small effect. The direct gene analysis approach enables the role of functional polymorphisms "candidate alleles" to be evaluated even under models of high genetic heterogeneity, and is also the logical follow up to whole genome linkage studies.

Because alcoholism is both common and etiologically complex, our linkage studies focus on two populations that are relatively homogeneous genetically and environmentally: American Indian and Finn. For the same reasons, we are also using neurophysiological and neurochemical traits which may identify genetically distinct subgroups. These include Caucasian and American Indian families with the low voltage alpha (LVA) EEG trait and Finnish families with low CSF 5HIAA probands. Alcohol sensitivity, personality traits and comorbid diagnosis are other subgroup-defining phenotypes available in various datasets.

The genetics of alcoholism also includes interindividual differences in sensitivity to alcoholism, including its deleterious consequences. Our research in this domain has been directed toward mechanisms of differential sensitivity to acute intoxication, to mechanisms and of DNA damage and damage prevention, and towards the control of expression of genes important in alcoholism vulnerability (genes such as tryptophan hydroxylase (TPH) and the serotonin transporter).

FAMILIALITY AND GENETIC LINKAGE OF ALCOHOLISM IN AMERICAN INDIANS

Alcoholism and its consequences are pervasive in most American Indian populations. For the development of treatment and prevention strategies, it is vital to establish the role and identity of causative factors in these populations, which also offer the advantage of greater genetic and environmental homogeneity than the U.S. population as a whole.

Despite the magnitude of the alcoholism problem in American Indians, neither a genetic transmission study nor a genetic linkage study had ever been performed. Also, the systematic data on the phenotype needed to address speculations about the reliability and meaning of the alcoholism diagnosis in American Indians largely derived from one other group of investigators. Whether Indian alcoholics were mis-classified binge drinkers was still an open question.

A large family (N=582) comprising a sizeable fraction of a Southwestern American Indian tribe with a high rate of alcoholism (85% of males, >50% of females) was systematically interviewed, evaluated for familial transmission of alcoholism and then tested, in collaboration with investigators from NIDDK (W. Knowler, R. Hanson, P. Bennett), for genetic linkage using a large (N=517) panel of DNA markers which covered all human chromosomes except the sex chromosomes: X and Y. The results, including findings on the meaning and consequences of alcohol-related diagnoses, are representative of the tribe. This is because (1) the family was ascertained solely on the basis of structure and availability, and (2) the family is representative of the overall tribal population in coefficient of relationship and in its demography.

In this Southwestern tribe, almost all of the large fraction of the population who were binge drinkers were also alcoholics and binge drinkers tended to become alcoholic at a younger age. Furthermore, regardless of whether binge drinkers met criteria for alcoholism, they were dramatically worse off in the four symptom categories evaluated: social, work, violence/lawlessness and physical. Analysis of psychiatric comorbidity revealed the same heavy clustering of psychiatric disorders with alcoholism as observed in the general population of the U.S., in studies such as the National Comorbidity Survey. These results may help to put to rest the misconception that drinking, particularly binge drinking, whatever its origin, is other than deleterious to American Indian communities.

We identified moderate to strong evidence for two potential alcoholism-vulnerability genes in this community. Significant and progressively declining risk for alcoholism was seen in females who were at the 1st, 2nd and 3rd degrees of genetic relationship to a female alcoholic. This corresponds to a heritability (role of genotype in vulnerability) of about 40%, similar to the general U.S. population. The same trend was present in males, but non-significant, perhaps due to the very high rate of alcoholism in males from this particular population.

The strongest evidence for an alcoholism-vulnerability gene was at the chromosome 11p telomere, directly at the location of the dopamine DRD4 gene that has been previously implicated in impulsive behavior. On a conservative basis, this statistical result could have been observed randomly in 1 in 6 whole genome scans, emphasizing the need for replication and extension of these results. This finding has been replicated in a second group of sib pairs derived from the same family, and, in addition, association is observed to a functional variant of the DRD4 gene. A moderate to strong linkage signal was also found on chromosome 4p, at the location of a cluster of GABAA receptor genes. These results are convergent with extensive pharmacological evidence (e.g., the cross-tolerance between alcohol and drugs which act at these receptors) and some previous animal genetic evidence implicating GABAA receptors in alcoholism. Finally, there was moderate evidence from this whole autosome scan for linkage of alcohol dependence to the chromosome 4q region that contains the alcohol dehydrogenase genes. This result is convergent with the recently established role for variants of alcohol dehydrogenase genes in alcoholism risk.

Current directions for linkage studies in American Indians are: 1) a linkage study in a low-alcoholism tribe, led by Dr. J. Long in collaboration with B. Albaugh (Center for Human Behavior Studies); 2) an EEG linkage study in a tribe with a relatively high rate of alcoholism; and 3) the Ten-Tribe Study, a genetic epidemiological study comparing tribes with low and high rates of alcoholism to identify gene-environment interactions (in concert with M. Koss, U Arizona). These studies are partially supported with funds from the Minority Health Initiative and data collections are by peer-reviewed contracts. The EEG study primarily involves neuropsychological assessment, of families we have previously studied, utilizing additional interviews and blood samples. Data collection is now complete in this family approximating 400 members. The Ten-Tribe Study (N=3000) investigates interaction of environment and genotype on differing rates of alcoholism as well as personal and community-wide effects of alcoholism, including trauma, in tribes with low and high rates of alcoholism. The differences in alcoholism rates between tribes are thought, on the basis of local health statistic data, to be dramatic, but have not been systematically documented. In the Ten-Tribe Study, 300 demographically sampled subjects from each of ten tribes are being psychiatrically interviewed (AUDADIS) and a blood sample is being collected for DNA analysis. The study is now complete in four of the target populations.

IDENTIFICATION OF VARIANTS OF CANDIDATE GENES FOR ALCOHOLISM AND OTHER BEHAVIORS AND LINKAGE TO BEHAVIOR

Among neurotransmitters, serotonin is important for its roles in behavioral inhibition and anxiety. Serotonin is thought to have an involvement in diverse disorders including schizophrenia, autism and alcoholism. Dopamine, endogenous opioids and GABAA receptors are critical for drug-mediated reinforcement and for other behaviors. A key step toward progress in understanding the genetics of complex diseases, such as alcoholism, is the identification of functionally significant gene variants (alleles) which can alter the function of neurotransmitters such as these. We identified a substantial collection of such variants and are relating them to alcoholism and other behaviors.

With regard to serotonin, amino acid substitutions of the serotonin receptors, designated 5HT1A, 5HT2A and 5HT2C, were discovered which alter receptor function. Each of these receptors is thought to be an

important pharmacological and natural target of action in serotonin function and two of these variants are common in the general population of the United States. The 5HT1A is one of two autoregulatory serotonin receptors and 5HT2A and 5HT2C are possible sites of action of the atypical antipsychotic drug, clozapine. The 5HT2A and 5HT2C variants have already been related to clozapine response, however, there are also contradictory results on this question and more data will be required. Therefore, these functional variants may provide important clues to the origins of natural behavioral variation, including psychiatric disease.

Recently we discovered common amino acid substitutions in several other receptors critical for understanding alcoholism. One of these is a common polymorphism (variant) of the mu opioid receptor, found by A. Bergen et al. Kreek and colleagues recently presented evidence that this receptor variant is functional. This receptor, thought to be involved in both pain and reinforcement, is a major site of action of the drug, naltrexone, which is showing promise in the treatment of alcoholism. We (N. Iwata et al.) also found a common amino acid substitution of the GABAA a6 receptor, the same gene that, in a rodent model, confers susceptibility to alcohol sensitivity. Studies are underway to relate these genetic variants to human behavior.

LINKAGE OF CANDIDATE GENE VARIANTS TO BEHAVIOR

A critical test of the DRD2 dopamine receptor, "Reward Deficiency Gene" hypothesis, was performed in a very large Southwestern American Indian linkage dataset. The linkage study was done with three DRD2 markers, including an amino acid substitution - Cys311, which dramatically impairs signal transduction by the activated receptor and which is far more abundant in this particular population as compared to Caucasians. There was neither genetic linkage nor genetic association to the dopamine receptor markers, including the functional amino acid substitution, suggesting that the DRD2 "Reward Deficiency Gene" hypothesis may be laid to rest.

An association of a genetic variant of tryptophan hydroxylase (TPH) to suicide was detected. TPH is rate limiting for the synthesis of serotonin, which is involved in impulsive behavior. In studies led by D. Nielsen, we were able report the replication of this association earlier this year, helping to solidify one of the first strong clues to the inherited biologic contribution to suicide.

Others have linked a functional genetic variant (5HTTLPR) of the serotonin transporter gene, the target of action of serotonin reuptake inhibitors used in the treatment of anxiety and depression, to anxiety and neuroticism. In a large study on sib pairs, collected at the University of Helsinki, C. Mazzanti et al. were able to replicate the linkage to anxiety reported by Lesch and colleagues. The HTTLPR variant may have diverse effects on behavior and, working with investigators at the NIMH, C. Mazzanti et al. have reported that it is predisposing to seasonal affective disorder (SAD) and the clinical presentation of schizophrenics--specifically, their degree of psychoticism.

The 5HT2A receptor is involved in appetite threshold and was reported to be associated with anorexia nervosa. We replicated this association in two populations of anorexia patients but found that bulimia patients do not show an association to this gene. Because anorexia patients differ from bulimics in having obsessional and perfectionist behaviors, we extended these 5HT2A studies to obsessive-compulsive disorder where a significant association was found.

We have built onto findings from mouse genetic models to identify a role for the 5HT1B receptor in a subtype of alcoholism. Use of animal genetic models to identify candidate genes is a key strategy in the analysis of complex diseases. Crabbe et al. found Quantitative Trait Locus for alcohol preference at the location of the 5HT1B receptor, the autoreceptor on serotonin nerve terminals. Later, this same group found that mice knocked out for 5HT1B drank more alcohol [this mouse phenotype is now known to be variable], and these mice were previously reported to be aggressive (Heu et al). Following up on the mouse results, we studied human alcoholics whose behavior was most likely to be homologous: alcoholics who also had antisocial personality or intermittent explosive disorder. Sib pair linkage analysis, using a marker we had found directly within 5HT1B and a flanking marker, was performed in both the Southwestern Indian and Finnish populations. Linkage was detected in both. The 5HT1B gene is located at about 80 cm from the 6p telomere. Interestingly, the COGA group has found a moderately positive linkage for the low amplitude P300 event-related potential to this same region of chromosome 6. This is a

convergent finding because this brain electrophysiologic trait is associated with alcoholism of the early onset, antisocial type.

NEUROPSYCHOLOGICAL PHENOTYPES

Neuropsychological markers for alcoholism vulnerability include the P300 event-related potential, the LVA EEG trait, sensitivity to acutely administered alcohol, personality measurements and neurochemical levels. All may identify genetically and physiologically more distinct subgroups of alcoholics as well as particular individuals that are at greater risk for alcoholism. Several other markers (low platelet monoamine oxidase and platelet adenylate cyclase) are found in a large fraction of alcoholics. These are more likely to represent long-lasting state changes associated with alcoholism and, therefore, were not selected as a focus for linkage studies.

The LVA EEG trait is an abundant, stable, neurophysiologic trait that appears to be transmitted in an autosomal dominant fashion. M-A Enoch previously reported a phenotype/phenotype association between LVA and alcoholism and recently replicated this observation. The frequency of LVA is about four times higher in alcoholics as compared to the general population and alcoholics with anxiety disorders are still more likely to have LVA. A linkage study, by Dr. Urbanek et al., failed to confirm the chromosome 20q linkage for LVA detected by Steinlein and colleagues. This result may be due to genetic heterogeneity. Our LVA genome scan did not detect linkage in this region. LVA is a relatively common trait and considerable genetic heterogeneity may be present, thus, reducing the power to detect linkage without large families. To generate large families individually capable of yielding significant linkage results, our EEG studies collected psychiatric diagnoses, cell lines, DNA and some genotypes on an American Indian family from a Plains tribe in Oklahoma. The pilot study (N=69) by M-A Enoch et al., detected LVA probands in five large pedigrees within what is essentially one very large tribal pedigree. This year we completed collection of neurophysiological phenotypes on over 400 members of this family. Linkage studies on this family will add to our ability to find alcoholism-vulnerability genes and to understand the gene/environment interaction in alcoholism vulnerability.

Schuckit and others have found that offspring of alcoholics have reduced sensitivity to various effects of ethanol and that this reduced sensitivity predicts greater vulnerability, regardless of family history of alcoholism. Differences in benzodiazepine sensitivity in offspring of alcoholics are more controversial (Cowley et al.), however, Korpi et al. found that a functional difference in the GABAA a6 receptor is probably responsible for some of the behavioral difference in alcohol preference between alcohol accepting and nonaccepting rat strains. This led us to study genetic variants of candidate genes associated with alcohol and benzodiazepine response in humans who had been carefully assessed for reaction to alcohol (in collaboration with M. Schuckit) and diazepam (in collaboration with D. Cowley). Alcohol response was correlated with genotype of the 5HTTLPR-serotonin transporter promoter polymorphisms and a novel, abundant amino acid substitution in the human GABAA a6 genotype (Iwata et al.). Although preliminary, these findings offer important clues as to the genetic basis of a trait-sensitivity to sedative-hypnotic drugs, which appears integral in vulnerability to alcoholism.

POPULATION GENETICS

The two focuses of population genetic research in LNG are: 1) the pattern of variation in populations of individual candidate gene polymorphisms whose origins and roles in behavior we need to better understand; and, 2) the genetic architecture of the Finnish and American Indian population isolates which are key to our genetic linkage studies. These studies have a direct connection to identifying genes for alcoholism. The choice of appropriate isolate populations and the use of tools of population and evolutionary genetics have enabled us to: perform meaningful simulation analyses to evaluate our genetic linkage (genome scan) findings; find evidence for selection for the ALDH2 Glu487lys polymorphism; and, find evidence for a role for a Y-chromosomal locus in alcoholism within the Finnish population.

ALDH2: The discoveries that the ALDH2 polymorphism, Glu487Lys, and the ADH2 polymorphism, Arg47His, affect alcoholism vulnerability are salient achievements of the field that encourage attempts to identify additional vulnerability alleles. Existence of Lys487/Lys487 homozygotes with very low or nonexistent ALDH2 enzyme activity raises questions as to the natural role of the functional enzyme, the

forces that have brought the nonfunctional 487Lys allele to high frequency in East Asia and the consequences of Lys487 for alcohol preference and response. The evolutionary origin of Lys487, including the timing of its origin (coalescence time) in human populations, was defined in our lab (R. Peterson et al.) by identifying multiple unique sequence variants at ALDH2 followed by a cladistic analysis across multiple populations. The results are consistent with a single origin of Lys487 for the modern populations studied, for a relatively ancient origin, and for an influence of selection.

Genetic variation and population history - autosomal: Studies on autosomal genetic variation (J. Long et al.) revealed that the American Indian populations we tested showed moderately reduced heterozygosity across a large panel of highly informative, short-tandem, repeat markers as compared to the cosmopolitan Caucasian population, although Finns, a well-defined population that is considered to be an isolate, did not show such a reduction in diversity. This study is the first to provide evidence that the population history of American Indians, including the dramatic 19th Century reductions in their numbers, has left a genome-wide imprint on diversity.

Genetic variation and population history - Y chromosome and mitochondrial: Our studies (R. Kittles et al.) have revealed that although autosomal diversity in Finns was unperturbed, the unique genetic history of Finland has left its imprint on Y chromosome diversity and haplotype pattern. In contrast to autosomal and mitochondrial DNA variation, Y haplotype diversity is greatly reduced, indicating a bottleneck effect specific to males (potentially the Thirty-Year War in which Finland was depopulated of males who fought in the Swedish army). In addition, the conception of Finland as an isolate population is seriously modified by our finding (Kittles et al., AJHG 1998) that this population has a dual origin from migrants who arrived earlier from Asia and later from Estonia.

Integration of population and behavioral genetics - Y chromosome association to alcoholism in Finns: R. Kittles et al. (PNAS, in press) used the knowledge we developed on Y chromosomal lineage in Finland to objectively group haplotypes for behavioral genetic association studies. Using methods originally developed by A. Templeton, Kittles et al. found that a specific Finnish Y chromosomal clade was associated with risk of alcoholism vulnerability. These particular Finnish Y chromosomes are, therefore, important targets for screening studies on the small number of genes which are found on the Y chromosome and which can potentially affect behavior.

GENE EXPRESSION

Tryptophan Hydroxylase (TPH): From heritability studies in humans and in rhesus macaque monkeys (Higley et al.), it is known that variation in serotonin function and, more precisely, CSF 5HIAA is substantially heritable. However, defined environmental influences, e.g., isolation or change in dominance status, are capable of influencing serotonin function and consequent behaviors. Therefore, it is important to elucidate the molecular and cellular adaptive mechanisms by which function is altered. One key target is the promoter for the gene for TPH, since this is the rate-limiting enzyme in serotonin synthesis. Dr. Nielsen elucidated the role of particular TPH promoter elements (four Sp1 sites and an RBP-Jk site). Working with A. Rotondo, he also identified several polymorphisms in the TPH promoter region that have been used for haplotype association. These variants have now been transfected into mammalian cells to explore their influence on transcription.

Tyrosine Hydroxylase (TH): The rate-limiting enzyme in catecholamine synthesis is TH. Within intro 1, this gene contains a repeat sequence capable of altering TH transcription (Meloni et al). The repeat is extremely polymorphic and one of the alleles has been reported to be associated with schizophrenia. Recently, we (Mazzanti et al.) have been able to show that different TH repeat alleles function differently in EMSA protein-DNA binding assays. These alleles are being transfected to investigate whether TH transcription is altered and, in addition, linkage studies in schizophrenia, alcoholism, and other conditions are under way.

DNA and protein damage and repair: Oxidative damage to DNA occurs due to alcohol intake. Dr. Brooks et al. established the presence of four DNA repair pathways in adult brain. It is possible that individuals with functional genetic variation in these pathways may be at greater vulnerability for alcohol-associated damage to nuclear DNA which, under ordinary circumstances, is largely repaired. This hypothesis is being explored in gene knockout mice. It is thought that a crucial step in the process of alcohol-associated protein and DNA-damage is the induction by alcohol of cytochrome P450 CYP2E1

leading to the generation of free radical metabolites and reactive oxygen species. Lipid-peroxides, such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA), are directly produced by CYP2E1 and are highly cytotoxic, probably because they directly react with proteins and DNA. For these reasons, Dr. Brooks has developed cell lines, deficient in nucleotide excision repair but rich in CYP2E1 activity, by using a retroviral vector containing CYP2E1 obtained from Dr. Arthur Cederbaum, Mount Sinai.

Toward understanding the role of the serotonin transporter in behavior, including alcohol intake, Drs. Ni, Brooks and colleagues have achieved transient transfection of neurons in tissue culture, and in the brain, using a HSV vector containing this gene. Very high levels of functional expression of the serotonin transporter and transfection efficiency were reached. This technique offers a unique opportunity to increase serotonin transporter function in specific brain regions and at particular points in development. Their studies have now moved forward to the microinjection of specific brain regions to be followed by neurochemical and behavioral studies.

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Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00008-07 LNG

Title: SEROTONERGIC GENE FUNCTION AND BEHAVIOR IN TRANSGENIC MICE

Principal Investigator: Goldman D, MD (MG, LNG, NIAAA)

Summary: TERMINATED

Former PI: David Nielsen, PhD
Section of Molecular Genetics, LNG, NIAAA

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00016-07 LNG

Title: GENE MAPPING AND LINKAGE STUDIES WITH SHORT TANDEM REPEAT (STR) MARKERS

Staff Years: 5.75

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Other Collaborators: Kittles RA, PhD (Microbiology, Howard University Cancer Center)

Sample Type: Interview, questionnaires or surveys and human tissues, fluids, cells, etc.

Keywords: gene mapping (human), neurosciences, drinking patterns/causes, molecular genetics

Summary: We are searching for genetic loci that contribute to the predisposition to alcoholism and related behaviors by conducting genetic linkage and mapping analysis using over 500 highly polymorphic DNA marker loci. These loci span all of the non-sex chromosomes at an average interval less than 10 centimorgans. To date, we have completed over 300,000 locus typings, primarily on American Indians and Finns. We have performed a whole autosomal genome scan for genetic linkage to alcohol dependence in a Southwestern American Indian tribe. The best evidence for linkage is seen with D11S1984 on chromosome 11p, in close proximity to several candidate genes with neurobiological functions. These candidate genes include the DRD4 dopamine receptor gene, the tyrosine hydroxylase gene and the tryptophan hydroxylase gene. Good evidence for linkage is also seen with D4S3242 on chromosome 4p, near the beta1 GABA receptor gene. The chromosome 11 findings were followed up in the past year by genotyping a high-resolution map on an expanded sample of subjects from the same Southwestern Indian population. The high-resolution genetic map includes polymorphisms within the DRD4 and tyrosine hydroxylase genes as well as STR markers closely linked to these candidate genes. We are also developing statistical approaches and software to identify the specific polymorphisms, from a set of closely linked loci, responsible for altering an individual's vulnerability to disease. So far, we have succeeded in identifying polymorphisms associated with disease status, but we have not identified specific variants altering vulnerability.

In Finns, we have completed much of a full genome search, tested for linkage to marker loci and to candidate genes, and tested for association with DNA markers on the Y chromosome. Our genome scan has revealed three chromosomal regions that are likely to harbor genes rendering vulnerability to alcohol dependence; one on chromosome 3, another on chromosome 5, and the last on chromosome 11. In addition, we have demonstrated association between Y-chromosome DNA markers and alcohol dependence and antisocial personality disorder. We find a significant association between alcoholism and 3 groups of Y-chromosomes that are closely related by their mutational histories. Interestingly, there is no association between Y-chromosomes and antisocial personality disorder after the comorbid effects of alcohol dependence have been removed. However, we find evidence for genetic linkage and association between antisocial personality disorder (ASPD) comorbid with alcoholism and the

chromosome 6, serotonin receptor gene, HTR1B. We also find evidence for linkage and association between ASPD with alcoholism and a polymorphism in the closely linked marker locus D6S286. These findings are confirmed by multipoint linkage analyses and by independent observation in the Southwestern Indian sample.

RESEARCH HIGHLIGHTS: The chromosome 11 findings were followed up in the past year by genotyping a high-resolution map on an expanded sample of subjects from the same Southwestern Indian population. We are also developing statistical approaches and software to identify specific polymorphisms responsible for altering an individual's vulnerability to disease and have succeeded in identifying polymorphisms associated with disease status, but not specific variants altering vulnerability.

Previously, we had demonstrated association between Y-chromosome DNA markers and alcohol dependence and antisocial personality disorder but we find there is no association between Y-chromosomes and antisocial personality disorder after the comorbid effects of alcohol dependence have been removed.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: It is well know that alcoholism is transmitted in families in a complex manner. Nonetheless, it has been difficult to identify either the contributing genes or specific environmental factors. This work has contributed to identifying the chromosomal regions that contain predisposing genes. Ultimately, we should be able to identify these genes and the mechanisms leading to alcoholism.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00017-07 LNG

Title: POPULATION GENETICS OF NATIVE AMERICAN TRIBES

Staff Years: 0.75

Principal Investigator: Long JC, PhD (PGL, LNG, NIAAA)

Other Personnel: Romero FC, MA (PGL, LNG, NIAAA)
Kolman C, PhD (PGL, LNG, NIAAA)
Lorenz J, PhD (PGL, LNG, NIAAA)
Rasmussen JK, MS (PGL, LNG, NIAAA)
High T, BS (PGL, LNG, NIAAA)

NIH Collaborators: Goldman D, MD (HN, LNG, NIAAA)

Other Collaborators: North C, MD (Indian Health Service)

Sample Type: Interview, questionnaires or surveys and human tissues, fluids, cells, etc.

Keywords: population research, ethnicity, molecular genetics

Summary: The purpose of this work is to ascertain the number of alleles, allele frequencies and allele frequency differences among American Indian tribes. The genetic systems being typed are the same as those being used currently in our genetic linkage analyses. Approximately 30 individuals from each of 20 tribes, collected primarily at the Albuquerque Indian Hospital between 1992 and 1994, are being analyzed. Various tests for allele frequency differences between tribal groupings, based on cultural and linguistic affinities, are being performed. In order to more fully quantify isolate structure, and exploit such populations for linkage analyses, we have developed a maximum likelihood method to characterize populations by their levels of gene identity. We have applied this method to microsatellite typings for three American Indian and three European populations. Low gene identity was observed in Europeans, approximately 28%. By contrast, gene identity was higher in all American Indian populations, about 39%. We have studied local patterns of gene diversity in 7 samples from populations in the Southwest and Alaska. These populations all speak closely related Athabascan languages, despite their dispersed geographic locations. When compared to non-Athabascan speaking neighbors, we do not find a strong tendency for these populations to comprise a unified gene pool. Rather, geographic proximity is the best predictor of the genetic relationship. In the past year, we have studied distribution in these populations of genetic polymorphism in unique sequences associated with genes that are related to neurobiology and alcohol metabolism. We have also been examining these polymorphisms in native Asian populations. This information is important to genetic linkage and disease association studies on American Indians because failure to account for population diversity can result in false evidence for linkage and allelic heterogeneity among groups can create spurious associations with disease.

RESEARCH HIGHLIGHTS: Previously, we reported that genetic diversity in highly polymorphic short tandem repeat loci is reduced by about 10% in Native Americans relative to European Americans. Also, that genetic relationships among tribes are better predicted by geographic proximity than by sharing of culture or language. In the past year, we studied distribution in these populations of genetic polymorphisms in unique sequences associated with genes that are related to neurobiology and alcohol metabolism. We also examined these polymorphisms in native Asian populations.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: This research shows that genetic diversity is not evenly distributed among members of the US population. The occurrences of certain genetic markers cluster in regional populations or ethnic groups such as Native Americans. The uneven distribution of diversity in populations can create false associations between marker genes and hereditary disorders. Knowledge of the patterns of diversity can help in the implementations of population genetic studies of disease.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00019-07 LNG

Title: ALDH2 DEFICIENCY--POPULATION GENETICS AND RELATIONSHIP TO PHENOTYPE

Staff Years: 1.2

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Xu K, MD, PhD (HN, LNG, NIAAA)
Akhtar L, MS (HN, LNG, NIAAA)
Schamberger SG (HN, LNG, NIAAA)
Enoch M, MD (HN, LNG, NIAAA)

NIH Collaborators: Long JC, PhD (PGL, LNG, NIAAA)
Nutman TB, MD (LPD, NIAID)
Zimmerman P, PhD (LPD, NIAID)

Other Collaborators: None

Sample Type: Human Tissues, Fluids, Cells, etc.

Keywords: population research, ethnicity, gene mapping (human), molecular genetics, drinking patterns & causes

Summary: In Orientals, ALDH2 deficiency due to a common polymorphism frequently causes a flushing reaction after alcohol consumption and this aversive reaction is responsible for lower rates of alcoholism in individuals with the inactive ALDH2-2 allele. ALDH2 deficiency was detected in South American Indian populations; however, these findings have never been confirmed and a previous search for the Oriental ALDH2-2 allele in South American Indians was negative. R. Peterson identified a series of additional markers at ALDH2, by restriction enzyme analysis, SSCP and sequencing haplotypes characteristic for the ALDH2-2 and ALDH2-1 alleles. This analysis has shed light on the origins and functional role of the variant ALDH2-2 allele that seems to have appeared on a single haplotype lineage and spread among East Asian populations. Although ALDH22R [Glu487Lys] probably originated on a single genetic background, haplotype analysis reveals that it is sufficiently ancient for additional mutations to have occurred subsequently. The results on ALDH2 haplotypes are most compatible with an effect of selection to maintain the Oriental ALDH2 variant, Glu487Lys. We are investigating the relationship of ADH and ALDH functional alleles to alcoholism and other substance abuse phenotypes. These genotype/phenotype relationships are being studied in combination with other loci, for example the OPRM1 receptor locus in opioid addiction.

RESEARCH HIGHLIGHTS: We have shown, through the analysis of ALDH2 haplotypes (constellations of genetic markers), that the functional ALDH2 variant causing the common enzyme deficiency in Orientals is ancient in origin and has probably been brought to high frequency by natural selection.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Evidence for selection to maintain a high frequency of the ALDH2 variant strongly implies that it has a role in physiology, even in the absence of alcohol intake.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00025-03 LNG

Title: TRANSMISSION AND GENETICS OF ALCOHOL DISORDERS IN A NATIVE AMERICAN TRIBE

Staff Years: 0.75

Principal Investigator: Long JC, PhD (PGL, LNG, NIAAA)

Other Personnel: Moore E (PGL, LNG, NIAAA)
Rasmussen JK, MS (PGL, LNG, NIAAA)
High T, BS (PGL, LNG, NIAAA)

NIH Collaborators: Goldman D, MD (HN, LNG, NIAAA)

Other Collaborators: Albaugh B, MSW (Center for Human Behavior Studies)

Sample Type: Interview, questionnaires or surveys and human tissues, fluids, cells, etc.

Keywords: gene mapping (human), neurosciences, drinking patterns/causes, molecular genetics

Summary: Using a whole genome scan, we will assess genetic linkage to alcoholism and associated psychiatric disorders in Choctaw American Indians. Choctaw is a large Eastern North American Indian tribe with over 30,000 enrolled members living within tribal boundaries in Oklahoma. By contrast to neighboring American Indian tribes that have high prevalence of alcoholism, this tribe stands out because alcoholism has a low prevalence, about 1% and 10% of females and males, respectively. By studying American Indians in the context of low alcoholism, we can expect to reveal different insights into the roles of genetic and/or environmental determinants of alcoholism. Genetic analysis is to be conducted using three samples from the tribe: a small random sample (N=100), 3 large extended families (N>80 per family), and a sample of more than 150 admixed nuclear families. The admixed nuclear families will be selected to have some Euro-American ancestry and at least one alcoholic family member. The transmission/disequilibrium test (TDT) is the linkage analysis method of principal interest, because of its demonstrated increased power with population admixture. However, the sampling design will also accommodate standard non-parametric two- and multi-point linkage methods. In order to perform the analyses outlined above, individual psychiatric interviews and blood samples have been and continue to be collected. Research diagnoses are made from the psychiatric interviews and DNA for genotyping is being extracted from the blood samples. For the linkage analysis, we expect to type up to 2000 unique sequence DNA polymorphisms spanning the entire human genome, as the technology becomes available. In the past year, collection of the random sample and extended families has been completed. Database checks and epidemiological analyses are now under way.

RESEARCH HIGHLIGHTS: Previously, we documented a Native American tribe with a low prevalence of alcoholism and related mental disorders. During the year, collection of the random and extended families samples was completed. Database checks and epidemiological analyses are now in progress.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: There are many genetic and environmental factors that contribute to risk for alcoholism. It may be possible to identify these factors by studying the transmission of alcoholism in semi-isolated communities such as Native American tribes. We can expect that each community will have fewer genetic and environmental factors contributing to risk and that these may be apparent by contrasting disease occurrence patterns in different communities.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00083-06 LNG

Title: DNA DAMAGE, DNA REPAIR AND ALCOHOL

Staff Years: 3

Principal Investigator: Brooks PJ, PhD (MN, LNG, NIAAA)

Other Personnel: Marietta CA, MS (MN, LNG, NIAAA)
Goldman D, MD (MN, LNG, NIAAA)
Momeni AK, BS (MN, LNG, NIAAA)
Huffman GN (MN, LNG, NIAAA)
Kang A (MN, LNG, NIAAA)
Lundsten K (MN, LNG, NIAAA)

NIH Collaborators: Robbins J, MD, PhD (DB, NCI)
Amin N, PhD (BNP, NINDS)
Tindall KR, PhD (ECM, NIEHS)
Sobol RW, PhD (LSB, NIEHS)

Other Collaborators: Cedarbaum A, PhD (Mt Sinai School of Medicine)
Van Steeg H, PhD (RIVM)

Sample Type: Neither human subjects nor human tissues

Keywords: neurosciences, molecular genetics, transgenic animals, cancer, cirrhosis, health & behavior

Summary: Alcohol consumption produces a variety of pathological effects, including fetal alcohol syndrome, liver and brain damage and an increased risk of certain types of cancers. The association between ethanol consumption and cancer suggests alcohol intake may adversely affect genomic DNA. Mechanisms by which ethanol can produce DNA damage are: 1) direct adduction of DNA by acetaldehyde, the major metabolite of ethanol or 2) the generation of DNA damaging oxygen radicals by cytochrome P450 2E1 (CYP2E1), which is induced by ethanol in liver and brain. To test the hypothesis, alcohol metabolism results in DNA damage, we created derivatives of the AS52 cell line expressing biologically relevant levels of CYP2E1 and ADH or both enzymes. AS52 cells contain a mutational target (the E. Coli gpt gene) that makes them highly sensitive for detecting mutations produced by cross-linking agent and oxygen radicals. Our prediction is that growing these cells in the presence of EtOH will increase mutation frequency. The level of DNA repair activity in target tissues is expected to be a crucial determinant of alcohol-induced DNA toxicity. Therefore, we have also placed the EtOH metabolizing enzymes in CHO cells that lack specific DNA repair pathways. This will allow us to identify which of the several DNA repair pathways play a role in protecting cellular DNA from genomic damage caused by EtOH metabolites. Analogous studies are being carried out in mice to assess whether the lack of specific DNA repair pathways makes them more susceptible to EtOH-related tissue pathologies. Another major focus is on N2-ethyl deoxyguanosine, the major DNA adduct produced by acetaldehyde. This adduct is undetectable in normal liver but accumulates in the DNA of mice fed alcohol. We are assessing whether the N2-ethyl deoxyguanosine adduct is a substrate for DNA repair and, if so, what type of repair is involved. We are also assessing the mutagenicity of this adduct in mammalian cells.

RESEARCH HIGHLIGHTS: We have discovered that a specific DNA repair pathway called nucleotide excision repair (NER) plays an important role in protecting cells from DNA damage arising from alcohol metabolism. This repair pathway is also involved in protecting the developing fetus from the toxic effects of ethanol *in utero*. In the course of these studies, we have identified a specific type of oxidative DNA damage that is involved in neurodegeneration and may play a role in alcohol related neuronal loss in chronic alcoholics.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM: Our work on the effect of alcohol on the genome and the role of DNA repair in protecting the genomic from alcohol related DNA damage

represents a new line of investigation within the Institute. A complete understanding of the molecular mechanisms by which chronic alcohol intake damages cells may lead to better treatments for chronic alcohol disease. In addition, our results provide the impetus for novel therapies to protect genomic DNA from alcohol related DNA damage.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00086-06 LNG

Title: MOLECULAR STUDIES ON GENETIC VARIANTS OF TRYPTOPHAN HYDROXYLASE AND 5HT1A

Principal Investigator: Goldman D, MD (MG, LNG, NIAAA)

Summary: MERGED WITH PROJECT Z01-AA-000290
"MOLECULAR GENETIC STUDIES OF SEROTONIN FUNCTION"

Former PI: David Nielsen, PhD, Section of Molecular Genetics, LNG, NIAAA

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00087-06 LNG

Title: STUDIES ON DNA SINGLE STRAND CONFORMATION PREDICTION

Principal Investigator: Goldman D, MD (LNG, NIAAA)

Summary: TERMINATED

Former PI: David Nielsen, PhD, Section of Molecular Genetics, LNG, NIAAA

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00101-04 LNG

Title: MANIPULATION OF SPECIFIC PROTEINS INVOLVED IN ALCOHOL INTAKE AND BEHAVIOR

Staff Years: 1.33

Principal Investigator: Brooks PJ, PhD (MN, LNG, NIAAA)

Other Personnel: Ni Y, MD, PhD (MN, LNG, NIAAA)
Marietta CA, MS (MN, LNG, NIAAA)
Goldman D, MD (MN, LNG, NIAAA)
Momeni AK, BS (MN, LNG, NIAAA)
Huffman GN (MN, LNG, NIAAA)
Kang A (MN, LNG, NIAAA)
Lundsten K (MN, LNG, NIAAA)

NIH Collaborators: None

Other Collaborators: Hoffman BJ, PhD (Neuroscience Research, Eli Lilly Res Lab)

Sample Type: Neither human subjects nor human tissues

Keywords: neurosciences, behavioral research, health & behavioral research, molecular genetics

Summary: There is abundant evidence linking brain monoamine neurotransmitter systems to behaviors such as alcoholism, aggression, reward, and psychiatric states including depression and schizophrenia. The evidence consists of correlations between monoamine levels and psychiatric disease and behavior in humans and drug effects in humans and experimental animals. The next step is to link specific molecules with behavioral effects. Our approach to this issue is to use viral vectors to deliver constructs that will modulate the level of specific behaviorally relevant proteins *in vivo*. We have produced a defective herpes virus that overexpresses the rat serotonin transporter (5HTT). The 5HTT is the target for the serotonin specific reuptake inhibitors, e.g., fluoxetine (Prozac TM), and a candidate gene in psychiatric diseases. The 5HTT virus has been shown to be functional when injected into the rat brain. The biochemical and behavioral effects of overexpressing the 5HTT in the raphe nuclei are now being examined.

RESEARCH HIGHLIGHTS: We have developed a viral vector system to transfer behaviorally relevant genes into the rodent brain. This method will allow us to study the role of specific gene products in behaviors such as alcohol intake and animal models of psychiatric disease.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Our work has led to the initiation of collaborative efforts with other investigators with expertise in studying alcohol intake in genetically modifies rats and mice. This collaborative effort promises to lead to a better understanding of the neurochemical basis of alcohol addiction, and the role of specific gene products in this condition.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00234-17 LNG

Title: MOLECULAR STUDIES ON SEROTONERGIC GENE EXPRESSION

Principal Investigator: Goldman D, MD (MG, LNG, NIAAA)

Summary: TERMINATED

Former PI: David Nielsen, PhD, Section of Molecular Genetics, LNG, NIAAA

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00280-10 LNG

Title: GENETIC STUDIES OF EEG AND ERP TRAITS RELATED TO ALCOHOLISM

Staff Years: 1.3

Principal Investigator: Enoch M, MD (HN, LNG, NIAAA)

Other Personnel: Goldman D, MD (HN, LNG, NIAAA)
Harris CR, BA (HN, LNG, NIAAA)
Robin RW, PhD (HN, LNG, NIAAA)
White KV, ME (HN, LNG, NIAAA)

NIH Collaborators: None

Other Collaborators: Rohrbaugh J, PhD (Psychiatry, Washington University)

Sample Type: Interview, questionnaires, or surveys only

Keywords: health & behavior, neurosciences, drinking patterns & causes, electrophysiology/EEG, molecular genetics

Summary: Alcoholism is a common heterogeneous disease. Heritability has been established in both men and women, but as for other psychiatric diseases, it has proved difficult to map genes directly for reasons such as genetic heterogeneity, phenocopies, penetrance and expressivity, and polygenic effects. We have identified a trait-specific marker for alcoholism vulnerability, the low voltage alpha (LVA) EEG, a normal variant of the resting EEG in which the alpha rhythm is virtually absent. This phenotype is strongly heritable, trait-like, present in 4-11% of the population and accurately determined. We now have a complete data set, including EEG and ERP phenotypes, blind-rated DSM-III-R diagnoses, psychometric tests and DNA on 247 individuals. We recently replicated the association of LVA with a subtype of alcoholism with anxiety in a comparable sample of 149 unrelated individuals (Enoch et al 1999). LVA was found in 23% of alcoholics, 25% of subjects with an anxiety disorder and 56% of alcoholics with an anxiety disorder compared to 8% of the individuals without alcoholism or an anxiety disorder. In order to obtain sufficient power to map genes for alcoholism, the focus of this study has shifted to a Plains American Indian tribe that has a high prevalence of alcoholism. During the course of this year we performed EEGs and ERPs on 374 tribal members from large pedigrees. We have almost completed the data set of psychiatric diagnoses and DNA from these individuals. We will soon be in a position to start analyses for mapping genes for alcoholism. Formerly Titled "Genetic studies of the electroencephalogram and event-related potentials."

RESEARCH HIGHLIGHTS: We have succeeded in collecting a full data set of EEG/ERP analyses, psychiatric diagnoses, psychological testing and DNA on approximately 360 members of a Plains American Indian tribe, who came from several large families.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: We now have a very large data set on 360 individuals who are all inter-related. This will allow us to map genes for alcoholism and nicotine addiction.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00281-10 LNG

Title: MOLECULAR GENETIC STUDIES ON ALCOHOLISM IN AMERICAN INDIANS - SOUTHWESTERN TRIBE

Staff Years: 1

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Akhtar L, MS (HN, LNG, NIAAA)
Schamberger SG (HN, LNG, NIAAA)
Robin RW, PhD (HN, LNG, NIAAA)

NIH Collaborators: Knowler WC, MD, DrPH (DAE, PECRB, NIDDK)
Akhtar L, MS (HN, LNG, NIAAA)
Long JC, PhD (PGL, LNG, NIAAA)
Bennett PH, MB, FRCP (PECRB, NIDDK)
Hanson RL, MD (DAE, PECRB, NIDDK)
Robin RW, PhD (HN, LNG, NIAAA)

Other Collaborators: None

Sample Type: Human tissues, fluids, cells, etc.

Keywords: population research, rural health, neurosciences, health & behavior, molecular genetics, gene mapping (human)

Summary: To identify alcoholism vulnerability and protective genes, we collected and tested for linkage and pattern of genetic transmission families from American Indian populations. Such populations are relatively homogeneous genetically and environmentally. This report addresses an American Indian tribe in which alcoholism is highly prevalent. A total of 582 subjects from a large family genealogy were psychiatrically interviewed (SADS-L), blind rated for diagnosis and genotyped. An analysis (J. Long) of the familiality of alcoholism revealed a significant increase in relative risk in first-degree relatives of alcoholics. In females, the risk was highest in first-degree relatives, still significant at the 3rd degree of genetic relationship and compatible with a heritability of about 40%. Binge drinking in American Indians was found to be a behavior which is not, as has been alleged, benign or beneficial, but associated with dramatic increases in problems in multiple domains: social, violence/lawlessness, work and medical. A whole autosome genetic linkage analysis on a subset of the sample utilized 517 short tandem repeat markers. By sib-pair analysis, strong evidence for linkage to alcohol dependence [DSM-III-R] was found near the chromosome 11p telomere [near the DRD4 dopamine receptor locus] and the centromeric region of chromosome 4p [near the GABA receptor cluster]. A more modest linkage signal was also detected on chromosome 4q [at the location of the alcohol dehydrogenase gene cluster]. Simulation analyses confirmed that these linkage signals were statistically highly significant. The 11p finding could, on a conservative basis, have been randomly expected in about one in six whole genome linkage analyses. The 11p linkage has been replicated in a second subset from the original population.

RESEARCH HIGHLIGHTS: Three putative genome locations for genes influencing alcoholism were identified, each at the location of a prominent candidate gene or genes. The locations were chromosome 11p – dopamine D4 receptor, chromosome 4p – GABAA receptor cluster and chromosome 4q – alcohol dehydrogenase gene cluster.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Identification of genes for alcoholism vulnerability can lead to new drug targets and more accurate targeting of therapies.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00290-09 LNG

Title: MOLECULAR GENETIC STUDIES OF SEROTONIN FUNCTION

Staff Years: 3

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Mazzanti C, PhD (HN, LNG, NIAAA)
Akhtar L, MS (HN, LNG, NIAAA)
Flores J (HN, LNG, NIAAA)
Schamberger SG (HN, LNG, NIAAA)

NIH Collaborators: Nielsen DA, PhD (MG, LNG, NIAAA)
Jenkins GL, MS (MG, LNG, NIAAA)
Higley JD, PhD (NN, LCS, NIAAA)
Long JC, PhD (PGL, LNG, NIAAA)
Weight FF, MD (LMCN, NIAAA)
Dean M, PhD (LVC, NCI)
Rosenthal NE, MD (CPB, NIMH)
Murphy DL, MD (LCS, NIMH)

Other Collaborators: Asberg M, MD (Department of Clinical Neuroscience, Karolinska Hospital)
Eggert M, MD (Psychiatry, University of Helsinki)
Johnson T (Institute of Behavior and Genetics)
Mann JJ, MD (Neuroscience, NY State Psychiatry Institute)
Pranzatelli M (Washington University)
Rotondo A, MD (Department of Psychiatry, University of Pisa)
Roy A, MD (Psychiatric Service, Dept Veterns Affairs Medical Center)
Siever LJ, MD (Psychiatric Service, Bronx VA Medical Center)
Virkkunen M, MD (University of Helsinki)

Sample Type: Human tissues, fluids, cells, etc.

Keywords: population research, neurosciences, gene mapping (human), molecular genetics, drinking patterns & causes

Summary: Studies on individuals and animals with genetic defects in serotonin function can shed light on the role of this neurotransmitter in behavior and on the role of milder functional variants in serotonin genes in predisposing individuals to psychopathologies and to alcoholism. We are identifying probands for family studies by measuring the serotonin metabolite 5HIAA in cerebrospinal fluid and by identifying individuals with amino acid substitutions in genes involved with serotonin function. Two 5HT1A variants are rare amino acid substitutions (Gly22Ser and Val28Ile), one conservative and one nonconservative. The 5HT2C variant is a common (allele frequency=0.18) nonconservative substitution (Cys23Ser). Two 5HT2A amino acid substitutions (Ala477Val and His452Tyr) have allele frequencies of 0.01 and 0.09. Rare serotonin transporter and 5HT7 amino acid substitutions were also discovered. Three of these amino acid substitutions were shown to alter the functional properties of the corresponding receptor. 5HT1A Gly22Ser when expressed in CHO-K1 cells dramatically altered desensitization and down regulation of these receptors. 5HT2C Cys23Ser in oocytes and COS-7 cells decreased ligand binding 5HT2A His452Tyr impaired signal transduction in platelets from subjects with the 452Tyr allele. For association and direct gene analysis, we collected more than 40 cell lines from each of the following populations: anorexia nervosa (collaboratively with W Kaye), obsessive compulsive disorder (D. Murphy), low CSF 5HIAA with Type II alcoholism (M Linnoila, M Virkkunen, M Eggert), and seasonal affective disorder (N Rosenthal, N Ozaki). The detected polymorphisms are converted to PCR RFLPs or allele-specific amplification markers for ease of analysis. Using the CEPH reference pedigrees and the polymorphisms at these genes, each gene is genetically mapped to its chromosomal location. For direct gene analysis, we mainly use single-strand conformational polymorphism (SSCP) analysis and direct sequencing. Association of a TPH polymorphism with suicidality in impulsive alcoholic Finns was

replicated. Sib-pair linkage of 5HT1B to antisocial alcoholism was found in Finns (J Lappalainen) and replicated in Southwestern American Indians. The serotonin transporter promoter variant, 5HTTLPR, which was previously linked to neuroticism was linked to the two anxiety related subscales of the Tridimensional Personality Questionnaire (TPQ) in a sib pair analysis (C Mazzanti), partially replicating an earlier finding. In a series of publications, we have shown that the 5HT2A-1438 G>A promoter variant is linked to anxiety related conditions. These include OCD, seasonal affective disorder, anorexia nervosa and anxiety-related scales from the TPQ.

RESEARCH HIGHLIGHTS: We have shown that the 5HT32A-1438G>A promoter variant is linked to anxiety-related conditions including OCD, SADS and anorexia nervosa.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: Anxiety is frequently a component in alcohol abuse. Identifying links to its causes may aid in identifying links to alcohol abuse.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00293-03 LNG

Title: ROLE OF THE SEROTONIN TRANSPORTER PROMOTER POLYMORPHISM IN PSYCHIATRIC DISORDERS

Staff Years: 3

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Mazzanti C, PhD (HN, LNG, NIAAA)
Bozak DJ, MS (HN, LNG, NIAAA)
Ellini A, BS (HN, LNG, NIAAA)
Sokolsky CL, BA (HN, LNG, NIAAA)
Vanakoski J, MD, PhD (HN, LNG, NIAAA)

NIH Collaborators: Long JC, PhD (PGL, LNG, NIAAA)
Rosenthal NE, MD (CPB, NIMH)
Murphy DL, MD (LCS, NIMH)

Other Collaborators: Cassano G, MD (University of Pisa)
Malhotra A, MD (Department of Psychiatry, Hillside Hospital)
Reist C, MD (University of California)
Rotondo A, MD (Department of Psychiatry, University of Pisa)
Virkkunen M, MD (University of Helsinki)

Sample Type: Interview, questionnaires, or surveys only

Keywords: neurosciences, health & behavior research, eating disorders, drinking patterns & causes, gene mapping (human), molecular genetics

Summary: Dysfunction in serotonergic pathways may underlie several psychiatric disorders. The serotonin transporter (5HTT) plays a critical role in the termination of serotonergic neurotransmission by Na-dependent uptake of serotonin by the presynaptic neuron. 5HTT also represents the initial site of action of certain antidepressant drugs and neurotoxins. A functionally significant polymorphism in the 5HTT promoter was identified (5HTTLPR). The polymorphism affects 5HTT transcription and, ultimately, 5HTT function. Frequency of the 5HTTLPR was determined in a variety of clinical psychiatric populations including alcoholics; linkage and association studies were performed. Positive linkage was detected between 5HTTLPR and the two anxiety-related personality traits available on the TPQ, at least partially replicating the reported association of this variant to behavior (see bibliography). In contrast, no association was found in Italian patients with obsessive-compulsive disorders, panic disorders and eating disorders. However, two additional disease-specific findings were made: 1) in collaboration with A Malhotra and D Pickar, 5HTTLPR was found to be significantly associated with BPRS-rated psychoticism in schizophrenia; and, 2) in collaboration with N Rosenthal, 5HTTLPR was significantly linked with seasonal affective disorder and seasonality rating in SAD patients.

RESEARCH HIGHLIGHTS: The 5HTTLPR promoter was identified in a variety of psychiatric populations including alcoholics.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: These discoveries may lead to identification of causes of alcoholism.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00294-03 LNG

Title: EVOLUTION AND VARIATION OF MACACA 5HT1A

Staff Years: 0.75

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Pratt ML, BA (HN, LNG, NIAAA)

NIH Collaborators: Nielsen DA, PhD (MG, LNG, NIAAA)
Jenkins GL, MS (MG, LNG, NIAAA)
Higley JD, PhD (NN, LCS, NIAAA)

Other Collaborators: Mehlman P, PhD (Laboratory Animal Breeders Service)
Raleigh M, PhD (University of California, Los Angeles)

Sample Type: Neither human subjects nor human tissues

Keywords: neurosciences, molecular genetics, gene mapping (nonhuman)

Summary: 5HT1A is the intronless coding locus (1266 base pair - 422 amino acids) for a G protein-coupled serotonin receptor with a typical 7-transmembrane structure, located on chromosome 5 in humans. Previous work in this laboratory discovered two variants (Biochem Biophys Res Commun 1995;210(2):530-6), characterized their frequency and distribution in human populations (Human Mutation 1996;7:135-43) and investigated their functional effects (Neuropsychopharmacology 1997; 17:18-26). In order to assess the polymorphic spectrum of this locus in a primate animal model heavily used in neuroscience research, we have cloned and sequenced the highly conserved 5HT1A gene from four macaque species (Macaca Fascicularis, Macaca Maura, Macaca Mulatta and Macaca Nemestrina) and from the vervet monkey (Cercopithecus Aethiops). Both interspecific and intraspecific sequence variations were discovered. The interspecific variation supports the known phylogeny of Macaca. The intraspecific variation will be characterized in a large group of Macaca Mulatta, for which serotonin metabolites and behavioral data exist, in order to assess potential association between serotonin receptor variants and behavior.

RESEARCH HIGHLIGHTS: We are examining Macaca Mulatta to assess potential associations between serotonin receptor variants and behavior.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: This may lead to identification of variants, which predispose to alcohol abuse.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00295-03 LNG

Title: Y CHROMOSOME POPULATION GENETICS

Staff Years: 2.25

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Aragon RA, BS (HN, LNG, NIAAA)
Radel MQ, MD (HN, LNG, NIAAA)
Bucceri MT (HN, LNG, NIAAA)

NIH Collaborators: Long JC, PhD (PGL, LNG, NIAAA)
Bergen A, PhD (NCI)

Other Collaborators: Hammond M, PhD (Natal Institute of Immunology)
Kidd J, PhD (Yale University)
Kidd K, PhD (Yale University)
Kittles RA, PhD (Microbiology, Howard University Cancer Center)
Klitz W, PhD (University of California, Berkely)

Sample Type: Human subjects

Keywords: population research, drinking patterns & causes, gene mapping (human), molecular genetics

Summary: The estimation of the mutation rates of Y chromosome microsatellite loci, human Y chromosome phylogeny and human population divergence dates are the goals of this project. Approximately 500 individuals, drawn primarily from 5 Asian and 11 Native American population samples, are being genotyped at 9 microsatellite loci and 5 non-repetitive loci with known ancestral state, all located on the non-recombining, non-pseudoautosomal region of the human Y chromosome. Haplotypes will be constructed from the collected genotypes and population genetic parameters including heterozygosity and allelic repeat unit variance statistics will be calculated to compare population diversities and haplotype/population associations. Phylogenetic analysis using distance (population variance) and parsimony (interhaplotypic distance) methods will construct networks of evolutionarily-related populations and haplotypes. Linkage disequilibrium statistics will be calculated to estimate microsatellite mutation rates using population model approaches adapted from autosomal linkage disequilibrium mapping methods. These analyses will contribute to an understanding of the dispersal and migration of ancestral Asian populations into Asia and the Americas and will describe the relationships among descendent populations through the combined population genetic, phylogenetic and linkage disequilibrium analyses performed.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00296-03 LNG

Title: ALCOHOL DEPENDENCE AND CHROMOSOME 11P15.5

Staff Years: 1

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: McKeane DP, BS (HN, LNG, NIAAA)
Robin RW, PhD (HN, LNG, NIAAA)

NIH Collaborators: Long JC, PhD (PGL, LNG, NIAAA)

Other Collaborators: None

Sample Type: Interview, questionnaires, or surveys only

Keywords: population research, neurosciences, health & behavior, gene mapping (human), molecular genetics, drinking patterns & causes, ethnicity

Summary: In order to follow-up a linkage finding on chromosome 11p15.5 to alcohol dependence (Long et al., submitted), two short tandem-repeat marker panels for semi-automated fluorescent genotyping containing 15 loci distributed over the distal 20 cM of chromosome 11p15.5 have been created. One panel (six loci) has been typed in approximately 500 psychiatrically interviewed individuals from a Southwest American Indian tribe. A second panel (nine loci) has been optimized and typing will begin soon. In addition, two coding polymorphisms and one promoter polymorphism at DRD4, a candidate gene for involvement in vulnerability to alcohol dependence, have been typed in order to develop haplotypes at this locus, distal to the short-tandem repeat locus linked in the whole genome linkage scan. Haplotype construction, sib-pair linkage analysis and map construction will be performed to confirm the primary linkage finding and define intervals of maximum linkage to alcohol dependence.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00297-03 LNG

Title: EVOLUTION AND VARIATION OF RPS4Y

Staff Years: 0.2

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: None

NIH Collaborators: O'Brien SJ, PhD (LVC, NCI)

Other Collaborators: Mehlman P, PhD (Laboratory Animal Breeders Service)
Park S, PhD (Seoul National University)
Smith K, PhD (Johns Hopkins University)
Tsai S, PhD (Taipei Blood Center)

Sample Type: Human subjects

Keywords: population research, ethnicity, molecular genetics, gene mapping (human)

Summary: Sequence variation within the non-pseudoautosomal region of the Y chromosome within and between the Hominidae can elucidate Y chromosome evolution and paternal human population history. The sequence of the coding region of the RPS4Y locus, a ribosomal protein gene, was determined in 4 non-human primate species and in 59 individuals from three human populations. Sequence analysis of RPS4Y in the Hominidae suggests that the RPS4Y protein is under relaxed functional selection compared to its highly conserved homolog, RPS4X, and predicts that the gene transposed to the Y chromosome approximately at the prosimian-simian divergence. Sequence variation at RPS4Y was detected both within and between human populations. One RPS4Y variant, C711T, appears to be the first common coding sequence polymorphism on the Y chromosome. The coalescent of human sequences (175,000 +/- 125,000 years) at this locus is similar to estimates derived from different Y loci and different human population samples. The ethnographic distribution of this Y chromosome substitution identifies a paternal lineage ancestral to Asian and Native American populations.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00298-03 LNG

Title: MU OPIOID RECEPTOR POLYMORPHISMS AND ALCOHOL DEPENDENCE

Staff Years: 0.2

Principal Investigator: Goldman D, MD (LNG, NIAAA)

Other Personnel: None

NIH Collaborators: Long JC, PhD (PGL, LNG, NIAAA)
Bergen A, PhD (NCI)
Dionne R (NIDCR)
Iadarola M (NIDCR)

Other Collaborators: Virkkunen M, MD (University of Helsinki)
O'Malley S (Yale University)

Sample Type: Interview, questionnaires, or surveys only

Keywords: drinking patterns & causes, craving/addictions, molecular genetics, gene mapping (human), nonalcoholic drug abuse & dependence

Summary: The mu opioid receptor is implicated in the reward, tolerance and withdrawal effects of alcohol and other drugs of abuse. We directly sequenced the human mu-opioid receptor locus, OPRM1, to detect natural variation that might affect the function of this receptor or be associated with psychiatric phenotypes related to opioid function. Four DNA sequence variants were found: three amino acid substitutions (Ala6Val [rare], Asn40Asp [frequency 10%], Ser147Cys [rare]) and one intronic variant (IVS2+691G/C [frequency 50%]). OPRM1 alleles, genotypes and haplotypes from three psychiatrically characterized population samples (N = 791) were used to perform association and sib-pair linkage analyses to alcohol dependence. There was no significant association or linkage between OPRM1 and alcohol dependence in any of the three population samples. These results and power calculations strongly suggest that variation at the mu-opioid receptor is not associated with vulnerability to DSM-III-R Alcohol Dependence. Variation is being investigated for possible association to response to opiate pharmacotherapy and to variation in opioid function, in collaboration with Stephanie O'Malley (Yale). A study on inherited differences in nociception has been initiated with Ray Dionne & Michael Iadarola (NIDCR, NIH). A large-scale, case-control association study on opioid addiction has recently been completed.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00299-03 LNG

Title: TRYPTOPHAN 2,3-DIOXYGENASE--CANDIDATE GENE FOR SEROTONIN METABOLISM DISORDERS

Staff Years: 0.33

Principal Investigator: Enoch M, MD (HN, LNG, NIAAA)

Other Personnel: Goldman D, MD (HN, LNG, NIAAA)
Harris CR, BA (HN, LNG, NIAAA)

NIH Collaborators: Murphy DL, MD (LCS, NIMH)

Other Collaborators: Cassano G, MD (University of Pisa)
Cook E, PhD (University of Chicago)
Kaye W, MD (Psychiatry, University of Pittsburgh Medical School)
Palmour R, PhD (McGill University)
Virkkunen M, MD (University of Helsinki)

Sample Type: Human tissues, fluids, cells, etc.

Keywords: behavioral research, health & behavior, neurosciences, molecular genetics, gene mapping (human), drinking patterns & causes

Summary: Genetic defects in the enzymes involved in serotonin metabolism may contribute to a wide range of neuropsychiatric diseases, from eating disorders, obsessive-compulsive disorder and alcoholism to autism. Tryptophan, obtained only from the diet in humans, is converted to serotonin by tryptophan hydroxylase or to kynurenine by tryptophan 2,3-dioxygenase (TDO2). Both enzymes are rate limiting in their respective pathways. The purpose of this study is to screen the TDO2 gene for polymorphisms, assess functionality, and search for disease associations in 350 individuals, primarily using single-strand conformational polymorphism (SSCP) analysis. Most of the coding region (11 of the 12 exons) and short regions of the introns were successfully amplified and screened across populations with anorexia or bulimia nervosa, obsessive-compulsive disorder, autism, major depression and suicidality, impulsivity and alcoholism, and subjects enrolled in a tryptophan depletion study. No associations were found for polymorphisms in introns 5, 6 and 11 nor for a variant in exon 7 (A to C, 749 Asn to His). In the SSCP screening of the promoter region, no polymorphisms were found in the regions of two TATA boxes. An A to C variant was detected in the putative glucocorticoid site but was not associated with disease. However, in the promoter region of GTT repeats, a GTT insertion was found which may be associated with impulsivity and novelty seeking but not with alcoholism. Three further polymorphisms have now been found in the promoter region and are in the process of being sequenced and screened across populations.

RESEARCH HIGHLIGHTS: The results of this study suggest that there may be some association between a variant in the promoter region of the enzyme, TDO2, and a type of novelty seeking or impulsivity in a population of Finnish alcoholics with anti-social personality disorder.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The results of this study are only preliminary and need to be verified.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00300-02 LNG

Title: 5HT2A RECEPTOR PROMOTER POLYMORPHISM, -1438G/A AND SEROTONIN DYSFUNCTION

Staff Years: 0.33

Principal Investigator: Enoch M, MD (HN, LNG, NIAAA)

Other Personnel: Goldman D, MD (HN, LNG, NIAAA)
Harris CR, BA (HN, LNG, NIAAA)

NIH Collaborators: Murphy DL, MD (LCS, NIMH)
Rosenthal NE, MD (CPB, NIMH)

Other Collaborators: Cassano G, MD (University of Pisa)
Kaye W, MD (Psychiatry, University of Pittsburgh Medical School)
Palmour R, PhD (McGill University)
Virkkunen M, MD (University of Helsinki)

Sample Type: Human tissues, fluids, cells, etc.

Keywords: genetics, serotonin receptor, association, anorexia nervosa, obsessive-compulsive disorder, depression

Summary: Brain serotonin dysfunction is implicated in a range of heritable diseases including alcoholism, mood disorders, eating and obsessive-compulsive (OCD) disorders. An important starting point for understanding innate vulnerability to such diseases is to identify variation in genes involved in serotonin function. The 5HT2A-receptor gene is thought to contribute to appetitive behaviors and to anxiety and is one site of action of antipsychotics, hallucinogens and anti-depressants. 5HT2A receptor densities are higher in individuals with depression and suicide attempts. In this study, we replicated an earlier finding of an association of the 5HT2A-promoter polymorphism -1438G/A with anorexia nervosa. In addition we showed that the association extends to OCD but not to bulimia nervosa, a disorder in which obsessive and perfectionistic traits are less manifest (Enoch et al 1998). We have now shown that the promoter polymorphism is associated with OCD in women but not men. We have also demonstrated that this 5HT2A polymorphism is associated with a particular personality type: low novelty seeking and high harm avoidance, again in women but not in men. We have shown an association of the -1438A variant allele with Seasonal Affective Disorder, a condition in which depression recurs in the winter and remits in the spring (Enoch et al 1999). Preliminary results show an association of this variant with major depression found in community populations but not with major depression found in populations with a high prevalence of severe alcoholism. Association studies are being completed in other data sets and preparations are being made for functional studies of this promoter polymorphism.

RESEARCH HIGHLIGHTS: We have replicated an earlier finding that a variant in the promoter region of the gene for the serotonin 2A receptor is associated with anorexia nervosa. We have also shown that it is associated with obsessive-compulsive disorder in women but not in men, and that it is associated with behavioral traits such as high harm avoidance and low novelty seeking which may underlie these disorders.

SIGNIFICANCE TO BIOMED RESEARCH AND TO THE PROGRAM: The results of this study suggests that the variant in this gene may predispose individuals to the development of the perfectionistic and obsessional type personality that underlies both anorexia nervosa and OCD but is not so manifest in bulimia. This may lead to more specific treatments for these disorders.

Dates: 10/01/1998 to 09/30/1999

Project Number: Z01 AA00301-01 LNG

Title: HIGH THROUGHPUT GENOTYPING AND GENOTYPE CALLS FOR SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

Staff Years: 3.7

Principal Investigator: Goldman D, MD (HN, LNG, NIAAA)

Other Personnel: Finnegan RW, BS (HN, LNG, NIAAA)
Rudolph J, PhD (HN, LNG, NIAAA)
Akhtar L, MS (HN, LNG, NIAAA)
Forsythe K (HN, LNG, NIAAA)
Garcia SA, BS (HN, LNG, NIAAA)
Lucas J, BS (HN, LNG, NIAAA)
Schamberger SG (HN, LNG, NIAAA)
Shenk LM (HN, LNG, NIAAA)
Vattikuti S (HN, LNG, NIAAA)

NIH Collaborators: None

Other Collaborators: None

Sample Type: Human Tissues, Fluids, Cells, etc.

Keywords: automated genotyping, single nucleotide polymorphisms

Summary: High throughput genotyping of SNPs promises to revolutionize our understanding of genetic variation and the ability to relate genetic variation to phenotypic variation, including disease for 3 reasons): (1) the number of SNPs which are potentially available, more than 300,000 across the human genome; (2) SNPs represent the majority of sequence variants which alter the structure and expression of gene products; (3) these variants are amenable to analysis by automated methods. We have implemented high throughput genotyping of SNPs using TaqMan type primer/probe methodology in which each allele in a biallelic polymorphism generates a different fluorescent signal. The resulting signals can be quantitated immediately following PCR [on an end-point multiwell fluorescent detector - the Cytofluor 4000] or even during PCR [using the PE 7700 which permits quantitation during each PCR cycle]. A critical issue is the ability to develop algorithms for automated allele calling. Using a set of readily genotyped and more problematically genotyped SNPs, we have made an advance over the available technology for automated genotype calling. We have used a K-means clustering algorithm that evaluates the ratio of the two fluorescent allele signals to make accurate genotype calls on data that would have needed manual evaluation.

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